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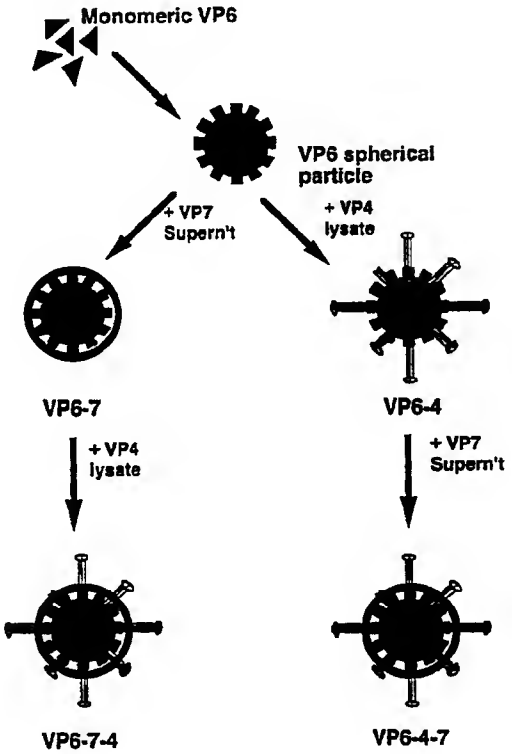

(57) Abstract

Assembled viral particles derived from rotavirus proteins are disclosed. The assembled particles include the inner capsid protein, VP6, in combination with either or both of the outer capsid proteins, VP4 and VP7. These assemblies can be used in vaccine compositions for the treatment and prevention of rotaviral disease.

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+ DESIGNATIONS OF "SU"

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ASSEMBLED VIRAL PARTICLES AND THEIR USE IN A VACCINE TO ROTAVIRAL DISEASE

Description

Technical Field

The present invention relates generally to virus-like particles which are useful as vaccines and immunogens. In particular, the instant invention concerns assembled rotaviral structural proteins and the use of the assembly in preventing and ameliorating rotaviral infection.

Background of the Invention

Rotaviruses cause gastrointestinal disorders and diarrhea in a wide variety of avian and mammalian species, including man. Several serotypes of rotavirus have been identified, four of which (serotypes 1 to 4) are found in humans and five of which (serotypes 3 to 7) are found in other animals. Recent studies indicate that cross protection among strains belonging to different serotypes may occur in animals including man. Ijaz et al., J Virol (1990) (In Press); Flores et al., J Clin Microbiol (1989) 27:512-518. The rotavirus genome is thought to consist of eleven segments of double-stranded RNA. The eleven genes encode the production of at least six structural proteins of the virus. In complete virus particles, these six proteins occur in a double-shelled arrangement. The outer shell or capsid is comprised of three proteins--virus protein 7 (VP7), virus protein 4

(VP4), and a third protein which has not yet been well characterized. There are three inner shell proteins designated virus protein 1 (VP1), virus protein 2 (VP2), and virus protein 6 (VP6).

VP7 is the major outer shell glycoprotein with an approximate molecular mass of 38 kD in its unreduced form (as determined by SDS-PAGE) and 42 kD in its reduced form (as determined by SDS-PAGE). VP7 has approximately 325 amino acids. The amino acid sequence of several rotavirus isolates has been determined and the sequences are approximately 75 to 86% homologous. Regions of conservation among human and animal species have been reviewed by Estes, M.K. et al., Microbiol Rev (1989) 53:410-449. This protein is known to bind to host cells (Sabara, M. et al., J Virol (1985) 53:58-66). Epitope mapping of VP7 using neutralizing monoclonal antibodies has localized a neutralizing-absorption domain to a component peptide with an approximate molecular mass of 14 kD (Sabara, M. et al., supra). Synthetic peptides derived from within this 14 kD fragment have also been shown to neutralize viral infectivity (Ijaz et al., supra).

A second outer capsid protein, VP4 (formerly designated VP3), is composed of 776 amino acids and has an approximate molecular mass of 82 kD in its unreduced form and 84 kD in its reduced form. The sequence of bovine VP4 has been determined (Potter, A.A. et al., Nucl Acid Res (1987) 15:4361) as has the partial amino acid sequence for simian VP4 (Mackow, et al., Proc Natl Acad Sci USA (1988) 85:645-649. VP4 possesses hemagglutinating activity, and induces the production of neutralizing antibodies which provide heterotypic passive protection in vivo (Offit, P.A. et al., J Virol (1986)

58:700-703). VP4 is responsible, in combination with VP7, in determining virus serotype.

Dimers of VP4 combine to form the surface spikes of rotavirus that extend distally from the rotavirus outer shell. VP4 is important in the penetration of the virus into the host cell and infectivity is increased by the cleavage of VP4 by trypsin. Trypsin enhanced infectivity is a common feature of all rotaviruses and the cleavage site for trypsin is also conserved as reviewed in Estes et al. (supra).

The inner capsid of rotavirus includes at least three proteins designated VP1, VP2 and VP6. Of interest herein is VP6 which is a 45 kD protein. Bovine VP6 appears to exist in trimeric units in both the virus particle and in infected cells, with the intersubunit linkage consisting of noncovalent interactions. These trimeric units complex further by virtue of disulfide bridges into larger units which likely represent the ring-like structures observed by several investigators using electron microscopy.

VP6 has been identified as the subgroup antigen and has also been described as the common rotavirus group antigen since some monoclonal antibodies raised against this protein react with all rotaviruses and polyclonal serum raised against a single rotavirus type can detect most other rotavirus strains. In addition to its antigenic properties, this nucleocapsid protein is extremely immunogenic and several investigators have found that the antibody raised to this protein has neutralizing ability. (See, e.g. Offit, et al., J Virol (1986) 58:700-703).

VP6 is an effective carrier protein (Redmond, M.J., et al. Mol Immunol (1990) (In Press) and VP4 is able to associate with VP6 monomeric and oligomeric

protein units. (Redmond, M.J., et al. supra). The VP4-VP6 association has been shown to withstand harsh treatment such as boiling in SDS. Additionally, VP6 is capable of forming particles in vitro with VP2 and VP7 using a calcium dependent process (Ready, K.F.M., et al., Virology (1988) 167:269-273). The resulting assembly is immunoreactive with antibodies specific for the whole virus as well as for immunodominant sites on VP6 and VP7 and this immunoreactivity is equivalent to that of native bovine rotavirus (BRV). (Ready, K.F.M., et al., supra).

The present invention provides assembled viral particles including peptides or proteins corresponding to VP7 and/or VP4, or immunogenic regions thereof, in combination with VP6. These assembled particles are effective as vaccines and in eliciting the production of neutralizing antibodies. Such a vaccine provides an alternative to the use of a live attenuated virus vaccine.

Disclosure of the Invention

The instant invention is based on the discovery that certain viral peptides, or epitopic regions thereof, when assembled into a viral particle, are able to elicit an immune response in a subject treated therewith. Vaccines including these assemblies are safer and more practical than those composed of attenuated virus.

Accordingly, in one aspect, the invention is directed to a viral particle assembly capable of eliciting an immunological response in a vertebrate subject. The viral particle assembly comprises:

(a) an inner capsid protein substantially homologous and functionally equivalent to VP6; and

(b) one or more outer capsid proteins selected from the group consisting of (i) a protein substantially

homologous and functionally equivalent to VP4, or a functional fragment thereof, and (ii) a protein substantially homologous and functionally equivalent to VP7.

In another embodiment, the present invention is directed to a viral particle assembly capable of eliciting an immunological response in a vertebrate subject wherein the viral particle assembly comprises VP6 assembled with VP4 and VP7.

In yet further embodiments, the invention is directed to vaccine compositions including a pharmaceutically acceptable vehicle and the viral particle assemblies described above.

In other embodiments, the instant invention is directed to methods of treating and preventing rotaviral disease in a vertebrate subject using the above vaccine compositions.

These and other embodiments of the present invention will readily occur to those of ordinary skill in the art in view of the disclosure herein.

Brief Description of the Figures

Figure 1 shows the nucleotide sequence and the predicted amino acid sequence of the VP6 protein of strain C486 (bovine).

Figure 2 compares the amino acid sequence of rotavirus VP6 derived from several strains: Bovine RF rotavirus (ROBMCP), human 1076 rotavirus (RO1HVP6), rotavirus segment 6 inner shell protein VP6 RNA (RO1S2VP6), equine H2 rotavirus (RO1VVP6H2), equine FI14 rotavirus (RO1VVP6F1), human Wa rotavirus (RO2SEG6), porcine Gottfried rotavirus (RO1PVP6), porcine group C rotavirus (PRVVP6), and simian SA11 rotavirus (ROTG6A).

Figure 3 compares the amino acid sequence of rotavirus VP4 derived from several strains: K8, KU, DS1, M37, ST3, SA11, and RRV.

Figure 4 shows the nucleotide sequence and corresponding amino acid sequence of the VP4 protein from strain C486 (bovine).

Figure 5 depicts the nucleotide sequence and corresponding amino acid sequence of the VP7 protein from strain C486 (bovine).

Figure 6 compares the amino acid sequence of rotavirus VP7 derived from several strains: simian 11 rotavirus (ROTVP7), rhesus rotavirus (RORVP7), porcine OSU rotavirus (PRVOSUVP7), human rotavirus (ROHVP7A), porcine Gottfried rotavirus (PRVPRVP7G), bovine uk rotavirus (ROB7), and porcine major C rotavirus (PRVPRVVP7).

Figure 7 is a graphic representation of the production of in vitro assembled rotavirus particles.

Detailed Description

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of immunology, protein chemistry, molecular biology, microbiology and recombinant DNA technology, which are within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Handbook of Experimental Immunology, Vols. I-IV (D.M. Weir and C.C. Blackwell eds., 1986, Blackwell Scientific Publications); Methods in Enzymology (S. Colowick and N. Kaplan eds., Academic Press, Inc.); Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual 2d Edition (Cold Spring Harbor Laboratory Press, 1989); Oligonucleotide Synthesis (M.J. Gait ed., 1984); and DNA Cloning, Volumes I and II (D.N. Glover, ed., 1985).

All patents, patent applications, and publications mentioned herein, whether supra or infra, are hereby incorporated by reference.

A. Definitions

In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

By "VP6" is meant the art-recognized major viral protein of the inner capsid from any species or strain within the family Reoviridae. See, e.g., Kapikian, et al., in Virology (B.N. Fields et al., eds., 1988). Examples of rotavirus strains from which the VP6 protein can be isolated and employed in the present invention include, but are not limited to, Simian SA11, human D rotavirus, bovine UK rotavirus, human Wa or W rotavirus, human DS1 rotavirus, rhesus rotavirus, the "O" agent, bovine NCDV rotavirus, human S2 rotavirus, human KUN rotavirus, human 390 rotavirus, human P rotavirus, human M rotavirus, human Walk 57/14 rotavirus, human Mo rotavirus, human Ito rotavirus, human Nemoto rotavirus, human YO rotavirus, human McM2 rotavirus, rhesus monkey MMU18006 rotavirus, canine CU1 rotavirus, feline Taka rotavirus, equine H2 rotavirus, human St. Thomas No. 3 and No. 4 rotaviruses, human Hosokawa rotavirus, human Hochi rotavirus, porcine SB2 rotavirus, porcine Gottfried rotavirus, porcine SB1A rotavirus, porcine OSU rotavirus, equine H1 rotavirus, chicken Ch.2 rotavirus, turkey Ty.1 rotavirus, bovine C486 rotavirus, and strains derived therefrom. Thus VP6 for use in the present invention includes VP6 from any rotavirus strain, whether from subgroup I, subgroup II, or any as yet unidentified subgroup, as well as from any of the serotypes 1-7, as well as any as yet unidentified serotypes.

The VP6 protein comprises an amino acid sequence of rotavirus which is unique to the class, or any member of the class, of VP6 polypeptides. A representative nucleotide sequence and the deduced amino acid sequence of bovine recombinant (BR) VP6 strain C486 is shown in Figure 1. Figure 2 shows the amino acid sequence of several different strains of rotavirus. As can be seen, extensive homology is present between the depicted rotavirus strains. Other VP6 nucleotide and amino acid sequences will find use in the instant invention, the depicted sequences only being representative of already known VP6 sequences.

By "VP4" is meant an art-recognized viral protein of the outer capsid from any species or strain within the family Reoviridae. Examples of rotavirus strains from which the VP4 protein can be isolated and employed in the present invention include, but are not limited to, those described above with reference to VP6.

VP4 (formerly designated VP3), is composed of 776 amino acids. The amino acid sequence of VP4 derived from strains K8, KU, DS1, M37, ST3, SA11, and RRV is shown in Figure 3. The nucleotide sequence and deduced amino acid sequence of VP4 from strain C486 (bovine) is shown in Figure 4. Again, other sequences will find use herein.

By "VP7" is meant the art-recognized major viral protein of the outer capsid from any species or strain within the family Reoviridae. Examples of rotavirus strains from which the VP7 protein can be isolated and employed in the present invention include, but are not limited to, those described above with reference to VP6.

VP7 is composed of approximately 325 amino acids and the nucleotide sequence and deduced amino acid

sequence of VP7 from strain C486 (bovine) is shown in Figure 5. Arias et al., J Virol (1984) 50:657-661 describes the nucleotide sequence of VP7 from simian SA11. Figure 6 depicts the amino acid sequences of several rotavirus strains. VP7 shows serotype restricted homology. The above sequences are meant to be representative and nonlimiting. Therefore, other functional sequences may also be employed with the present invention.

Two DNA or protein sequences are "substantially homologous" when at least about 85% (preferably at least about 90%, and most preferably at least about 95%) of the nucleotides or amino acids match over a defined length of the molecule. DNA sequences that are substantially homologous can be identified in a Southern hybridization experiment under, for example, stringent conditions, as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Sambrook et al., supra.

The term "functionally equivalent" refers to sequences of an analog of an outer or inner capsid rotavirus protein which define a chain that will produce a protein that elicits an immunological response equivalent to that elicited by the native sequence. Thus, the rotavirus proteins utilized herein need have the identical amino acid sequence of the native proteins.

A "functional fragment" of a rotavirus protein is a fragment with the capability of raising an immunological response equivalent to that elicited by the full sequence. It has been demonstrated that the distal end of VP4 is involved in the initial attachment of the virion to the cell, since infection may be blocked by monoclonal antibodies to this region. Furthermore, the enzyme trypsin enhances virus infectivity. This

enhancement appears to act after adsorption, since trypsin does not affect the efficiency or rate of virus attachment to cells but does increase the levels of uncoated particles found in cells. The molecular mechanism for trypsin enhanced infectivity occurs via the cleavage of the VP4 protein into two fragments with approximate molecular weights of 28 kDa and 60 kDa, respectively. Therefore, the trypsin cleavage site of VP4 is important in rotavirus replication. Thus, fragments consisting of an amino acid sequence substantially homologous to at least the first 255 N-terminal amino acids, as depicted in Figure 4, and which include the trypsin cleavage site, will also find use in the instant invention so long as these fragments are capable of raising an immunological response as defined above.

"A viral particle assembly" refers to an association between outer and inner capsid proteins of rotavirus, or proteins substantially homologous and functionally equivalent thereto, or functional fragments thereof. The particles can be assembled in vitro, as described more fully below, to resemble double shelled rotavirus.

The term "epitope" refers to the site on an antigen or hapten to which a specific antibody molecule binds. The term is also used interchangeably with "antigenic determinant" or "antigenic determinant site."

An "immunological response" to a composition or vaccine is the development in a vertebrate subject of a cellular and/or antibody mediated immune response to the composition or vaccine of interest. Usually, such a response consists of the subject producing antibodies, B cells, helper T cells, suppressor T cells, and/or cytotoxic T cells directed specifically to an antigen or

antigens included in the composition or vaccine of interest.

An "immunogenic protein" is a protein which elicits an immunological response in a subject to which it is administered.

The terms "polypeptide" and "protein" are used interchangeably herein and are used in their broadest sense, i.e., to denote any polymer of amino acids (dipeptide or greater) linked through peptide bonds. Thus, the terms encompass oligopeptides, protein fragments, analogs, mutants, fusion proteins and the like.

"Native" proteins or polypeptides refer to proteins or polypeptides recovered from rotavirus or from rotavirus infected cells. Thus the term includes naturally occurring rotavirus proteins and fragments thereof. "Non-native" polypeptides refer to polypeptides that have been produced by recombinant DNA methods or by direct synthesis. "Recombinant" polypeptides refer to polypeptides produced by recombinant DNA techniques; i.e., produced from cells transformed by an exogenous DNA construct encoding the desired polypeptide.

A "replicon" is any genetic element (e.g., a plasmid, a chromosome, a virus) that behaves as an autonomous unit of polynucleotide replication within a cell; i.e., capable of replication under its own control.

A "vector" is a replicon in which another polynucleotide segment is attached, so as to bring about the replication and/or expression of the attached segment. An "expression vector" refers to a vector capable of autonomous replication or integration and contains control sequences which direct the transcription and translation of the desired DNA sequence in an appropriate host.

A "coding sequence" is a polynucleotide sequence which is transcribed and/or translated into a polypeptide.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase and initiating transcription of a downstream (i.e., in the 3' direction) coding sequence.

A coding sequence is "under the control" of the promoter sequence in a cell when transcription of the coding sequence results from the binding of RNA polymerase to the promoter sequence; translation of the resulting mRNA then results in the polypeptide encoded within the coding sequence.

"Operably linked" refers to a juxtaposition wherein the components are configured so as to perform their usual function. Thus, control sequences operably linked to a coding sequence are capable of effecting the expression of the coding sequence.

"Control sequences" refers to those sequences which control the transcription and/or translation of the coding sequence(s); these may include, but are not limited to, promoter sequences, transcriptional initiation and termination sequences, and translational initiation and termination sequences. In addition, "control sequences" refers to sequences which control the processing of the polypeptide encoded within the coding sequence; these may include, but are not limited to sequences controlling secretion, protease cleavage, and glycosylation of the polypeptide.

A "signal sequence" can be included before the coding sequence. This sequence encodes a signal peptide, N-terminal to the polypeptide, that communicates to the host cell to direct the polypeptide to the cell surface or secrete the polypeptide into the media, and this

signal peptide is clipped off by the host cell before the protein leaves the cell.

A "host cell" is a cell which has been transformed, or is capable of transformation, by an exogenous DNA sequence.

A cell has been "transformed" by exogenous DNA when such exogenous DNA has been introduced inside the cell membrane. Exogenous DNA may or may not be integrated (covalently linked) to chromosomal DNA making up the genome of the cell. In procaryotes and yeasts, for example, the exogenous DNA may be maintained on an episomal element, such as a plasmid. With respect to eucaryotic cells, a stably transformed cell is one in which the exogenous DNA has become integrated into the chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eucaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the exogenous DNA.

A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth in vitro for many generations.

A "heterologous" region of a DNA construct is an identifiable segment of DNA within or attached to another DNA molecule that is not found in association with the other molecule in nature. Thus, when the heterologous region encodes a bacterial gene, the gene will usually be flanked by DNA that does not flank the bacterial gene in the genome of the source bacteria. Another example of the heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g., synthetic sequences having codons different from the native gene). Allelic variation or

naturally occurring mutational events do not give rise to a heterologous region of DNA, as used herein.

A composition containing A is "substantially free of" B when at least about 85% by weight of the total of A + B in the composition is A. Preferably, A comprises at least about 90% by weight of the total of A + B in the composition, more preferably at least about 95%, or even 99% by weight.

A "vaccine composition," according to the present invention, is an otherwise conventional vaccine formulation employing either the viral particle assemblies alone or in combination with one or more unassembled purified viral proteins or with cell lysates having one or more of the individual outer and/or inner capsid proteins. Particularly useful is the addition of a crude cell lysate containing VP4 to the instant vaccine compositions. The preparation of vaccines containing the above active ingredients is well understood in the art. Typically, vaccines are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified or the active ingredient encapsulated in liposomes. The active immunogenic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or adjuvants which enhance the effectiveness of the vaccine. The vaccines are conventionally administered parenterally, by injection, for example,

either subcutaneously or intramuscularly. Injectable vaccine formulations will contain an effective amount of the active ingredient, the exact amount being readily determined by one skilled in the art. The active ingredient can range from about 0.01% to about 95% (w/w) of the injectable composition, or even higher or lower if appropriate.

Additional vaccine formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulation. For suppositories, the vaccine composition will include traditional binders and carriers, such as polyalkaline glycols, or triglycerides. Such suppositories may be formed from mixtures containing the active ingredient in the range of about 0.5% to about 10% (w/w), preferably about 1% to about 2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium, stearate, sodium saccharin cellulose, magnesium carbonate, and the like. These oral vaccine compositions may be taken in the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations, or powders, and contain from about 10% to about 95% of the active ingredient, preferably about 25% to about 70%.

Furthermore, the viral particles may be formulated into vaccine compositions in either neutral or salt forms. If salts are used, the final preparation will typically contain less than 0.15M salt. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the active polypeptides) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric,

mandelic, and the like. Salts formed from free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylaminoethanol, histidine, procaine, and the like.

By "treating" is meant curing or ameliorating a subject that has contracted a rotaviral infection. "Preventing" rotaviral disease means preventing the occurrence of the infection, or tempering the severity of the infection if it is contracted subsequent to the administration of the instant compositions.

The vaccine composition of the present invention may be administered in a manner compatible with the dosage formulation, and in such amounts as will be therapeutically effective and immunogenic. A "therapeutically effective amount" of a vaccine composition is a dose sufficient to either prevent or treat rotaviral infection in a subject to which the composition is administered. The dosages of the viral particle assemblies which can treat or prevent rotaviral infection can be determined in view of this disclosure by one of ordinary skill in the art by running routine trials with appropriate controls. Comparison of the appropriate treatment groups to the controls will indicate whether a particular dosage is effective in preventing or treating a disease used in a controlled challenge. In general, effective dosage will vary depending on the mode of administration. For example, in the case of an intramuscular injection, generally from 0.001 μg/kg to 10 μg/kg will find use in the instant invention.

B. General Methods

Central to the instant invention is the discovery that assembled viral particles comprising rotavirus inner and outer capsid proteins, are able to elicit an immune response in a subject to which they are administered. The viral particles are assemblies of inner and outer capsid proteins of rotavirus. Particularly useful is a viral particle assembly including the inner capsid protein, VP6, with either or both of the outer capsid proteins, VP4 and VP7. VP6 appears to act as a "carrier" for the two outer capsid proteins, as described more fully below. These assemblies can be used alone, or in combination with unassembled, outer capsid proteins, provided as purified or partially purified proteins, or crude cell lysates, in a vaccine composition for the treatment and/or prevention of rotaviral infection.

The inner and outer capsid proteins for use in the viral particles of the instant invention can be prepared by any of several methods. First, the individual proteins can be isolated by successive degradation of purified virus with EDTA and either calcium chloride ($CaCl_2$) or lithium chloride (LiCl) treatment by standard techniques. See, e.g., Almeida et al., J Med Virol (1979) 4:269-277; Bican et al., J Virol (1982) 43:1113-1117; Gorziglia et al., J Gen Virol (1985) 66:1889-1900; Ready et al., Virology (1987) 157:189-198. Alternatively, the viral proteins can be produced by recombinant DNA techniques, which are fully explained in the literature. See, e.g., Sambrook et al., supra; and DNA Cloning, supra.

DNA coding sequences encoding the viral polypeptides can be derived from the particular mRNA. See, e.g., Estes et al., supra; Both et al., J Virol

(1984) 51:97-101; Cohen et al., Virology (1984) 138:178-182. Alternatively, a DNA sequence encoding the particular viral protein can be prepared synthetically rather than cloned. The DNA sequence can be designed with the appropriate codons for the viral protein amino acid sequence. In general, one will select preferred codons for the intended host if the sequence will be used for expression. The complete sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., Edge, Nature (1981) 292:756; Nambair et al., Science (1984) 223:1299; Jay et al., J Biol Chem (1984) 259:6311.

Once a coding sequence for the viral protein has been prepared or isolated, it can be cloned into any suitable vector or replicon. Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. Examples of recombinant DNA vectors for cloning and host cells which they can transform (in parenthesis) include the bacteriophage lambda (E. coli), pBR322 (E.coli), pACYC177 (E. coli), pKT230 (gram-negative bacteria), pGV1106 (gram-negative bacteria), pLAFR1 (gram-negative bacteria), pME290 (non-E. coli gram-negative bacteria), pHV14 (E. coli and Bacillus subtilis), pBD9 (Bacillus), pIJ61 (Streptomyces), pUC6 (Streptomyces), YIp5 (Saccharomyces), YCp19 (Saccharomyces) and bovine papilloma virus (mammalian cells). See generally, DNA Cloning: Vols. I & II, supra; and Sambrook et al., supra.

The coding sequence for the viral protein can be placed under the control of a promoter, ribosome binding site (for bacterial expression) and, optionally, an operator (collectively referred to herein as "control"

elements), so that the DNA sequence encoding the viral protein is transcribed into RNA in the host cell transformed by a vector containing this expression construction. The coding sequence may or may not contain a signal peptide or leader sequence. In bacteria, for example, the viral protein is preferably made by the expression of a coding sequence containing a leader sequence which is removed by the bacterial host in post-translational processing. See, e.g., U.S. Patent Nos. 4,431,739; 4,425,437; 4,338,397.

An expression vector is constructed so that the particular coding sequence is located in the vector with the appropriate regulatory sequences, the positioning and orientation of the coding sequence with respect to the control sequences being such that the coding sequence is transcribed under the "control" of the control sequences (i.e., RNA polymerase which binds to the DNA molecule at the control sequences transcribes the coding sequence). The control sequences may be ligated to the coding sequence prior to insertion into a vector, such as the cloning vectors described above. Alternatively, the coding sequence can be cloned directly into an expression vector which already contains the control sequences and an appropriate restriction site.

A number of procaryotic expression vectors are known in the art. See, e.g., U.S. Patent Nos. 4,440,859; 4,436,815; 4,431,740; 4,431,739; 4,428,941; 4,425,437; 4,418,149; 4,411,994; 4,366,246; 4,342,832; see also UK Patent Applications GB 2,121,054; GB 2,008,123; GB 2,007,675; and European Patent Application 103,395. Yeast expression vectors are also known in the art. See, e.g., U.S. Patent Nos. 4,446,235; 4,443,539; 4,430,428; see also European Patent Applications 103,409; 100,561; and 96,491.

Particularly useful for expression of the rotaviral genes of the instant invention are insect cells and vectors suitable for use in these cells. Such systems are known in the art, and include, for example, insect expression transfer vectors constructed from the baculovirus Autographa californica nuclear polyhedrosis virus (AcNPV). Such expression vectors typically use the strong viral polyhedrin gene promoter to control expression of heterologous genes. Methods for the introduction of heterologous DNA into the desired site in the baculovirus are known in the art. (See, e.g. Smith, et al., Mol and Cell Biol (1983) 3:2156-2165; and Luckow and Sumners Virology (1989) 17:31. Insertion can be, for example, into a gene such as the polyhedrin gene, by homologous recombination. Insertion can also be into a restriction enzyme site engineered into the desired baculovirus gene. Sequences encoding signal peptides can also be used in these expression systems since these peptides are recognized by insect cells and will cause the secretion of the expressed product into the expression medium.

Depending on the expression system and host selected, the viral protein is produced by growing host cells transformed by an expression vector described above under conditions whereby the protein is expressed. The protein is then isolated from the host cells and purified. If the expression system secretes the protein into growth media, the protein can be purified directly from cell-free media. If the protein is not secreted, it is isolated from cell lysates. The selection of the appropriate growth conditions and recovery methods are within the skill of the art.

Purified VP6 protein exhibits structural polymorphism. Specifically, hexamers and small hexagonal

lattices are present in many of the samples. Tubular particles form between about pH 5.0 and about pH 9.0, and are moderately stable to changes in temperature and ionic strength. The formation of these particles is fully reversible. Spherical particles reassembling single-shelled virus can be formed at about pH 4.0. A novel structure, in the form of sheets, composed of small-hole lattice, is formed in samples shifted from about pH 6.0 to about pH 4.0. These results demonstrate the importance of VP6 and of protein-protein interactions for rotavirus assembly.

Such protein-protein interactions are likely involved in the observed phenomenon that certain peptides can bind to VP6 in its monomeric form or to various oligomeric structures formed from VP6 monomers, such as in vitro assembled tubes and spheres. The attachment is mediated by a specific binding site(s) within VP6.

After the individual outer and inner capsid proteins have been either isolated, synthesized, or recombinantly produced, these proteins are assembled into viral particles using a calcium dependent process described in the examples and in Ready, K.F.M., et al., Virology (1988) 167:269-273. Figure 7 depicts a representative scheme for the production of in vitro assembled rotavirus particles.

As explained above, the viral particles can be administered in vaccine compositions in combination with purified, unassembled outer capsid proteins, or with cell extracts including one or more of the individual outer capsid proteins. These cell extracts are prepared by techniques well known in the art. For example, cell extracts can be prepared by cultivating rotavirus in African Green Monkey MA104 cells, harvesting the cells and media together and removing the cells by centri-

fugation. The resulting supernatant includes rotavirus proteins. The proteins can be further purified using density gradient ultracentrifugation. The virus pellet is then subjected to treatment with $CaCl_2$ and $LiCl_2$, as described in Ready, K.F.M. and Sabara, M. Virology (1987) 157:189-198, to obtain the individual viral particles. VP4 is present in very small amounts in such preparations. Furthermore, small amounts of VP4 are often present in the VP7 fractions.

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

Deposits of Strains Useful in Practicing the Invention

A deposit of biologically pure cultures of the following strains was made with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD. The accession number indicated was assigned after successful viability testing, and the requisite fees were paid. Access to said cultures will be available during pendency of the patent application to one determined by the Commissioner to be entitled thereto under 37 CFR §1.14 and 35 USC §122. All restriction on availability of said cultures to the public will be irrevocably removed upon the granting of a patent based upon the application. Moreover, the designated deposits will be maintained for a period of thirty (30) years from the date of deposit, or for five (5) years after the last request for the deposit; or for the enforceable life of the U.S. patent, whichever is longer. Should a culture become nonviable or be inadvertently destroyed, or, in the case of plasmid-containing strains, lose its plasmid, it will be

replaced with a viable culture(s) of the same taxonomic description.

Strain	Deposit Date	ATCC No.
pAC373BRV6 (in E. coli)	31 August 1987	40362
BVLVP4 (in A. californica)	24 October 1990	
BYVP7 (in A. californica)	24 October 1990	

C. Experimental

Materials and Methods

Enzymes were purchased from commercial sources, and used according to the manufacturers' directions. Radionucleotides and nitrocellulose filters were also purchased from commercial sources.

In the cloning of DNA fragments, except where noted, all DNA manipulations were done according to standard procedures. See, Sambrook et al., supra. Restriction enzymes, T_4 DNA ligase, E. coli, DNA polymerase I, Klenow fragment, and other biological reagents were purchased from commercial suppliers and used according to the manufacturers' directions. Double-stranded DNA fragments were separated on agarose gels.

Animals:

Rotavirus-free mice (CD1) were purchased from Harlan Sprague Dawley Inc., (Indianapolis, IN). The age of the mice at the time of purchase was approximately 6 weeks. The mice weighed between 25 and 30 grams. All mice were found to be seronegative for antibodies to rotavirus using an ELISA assay (Ijaz, M.K. et al. Exp Mol

Path (1989) 51:186-204). The animals were housed in isolation units throughout the experiment.

Cells and Virus:

MA104 cells (African green monkey) were cultured in Eagle's minimal essential media (MEM) supplemented with 10% fetal bovine serum (FBS) (Gibco Laboratories, Grand Island, NY). Bovine rotavirus isolate C486 was cultured from the feces of diarrheic calves by a method described previously (Babiuk, L.A. et al., J Clin Microbiol (1977) 6:610-617). Rotavirus C486 is publicly available from the ATCC, Rockville, MD (accession no. VR-917). Simian rotavirus strain SA11 (serotype 3) was obtained from Dr. H. Malherbe (San Antonio, TX) and human rotavirus strain DS1 (serotype 2) and human rotavirus strain Wa (serotype 1) were obtained from Dr. H. Greenberg (Stanford University, CA).

These viruses were propagated in confluent MA104 cells in the presence of 1 μg of trypsin (Difco Laboratories, Detroit, MI) per ml in the absence of FBS. Cells and supernatant were harvested together and cells were removed by centrifugation at 500g for 20 min. Virus was concentrated from the clarified supernatant fluids by pelleting through a 40% sucrose-cushion at 100,000 g for 2½ hr at 15°C. The virus pellet was resuspended in double distilled water and the amount of virus protein was estimated spectrophotometrically as described previously (Ijaz, M.K. et al., Antiviral Res (1987) 8:283-298). The resuspended virus was stored at -70°C. Individual inner and outer capsid proteins were isolated from the preparation as described in the examples below.

Plaque Assay:

A plaque assay for the quantitation of infectious rotavirus was performed according to the method described previously (Aha, P.M. and Sabara, M.I. J Virol (1990) 28:25-32). Briefly, 12 well tissue culture plates (NUNC) containing confluent monolayers of MA104 cells, were washed twice with MEM (without FBS). Serial tenfold dilutions of each rotavirus isolate were prepared in MEM containing trypsin (Difco Laboratories, Detroit, MI) to a final concentration of 10 μg/ml. Following adsorption of the virus at 37°C for 1 hr, the inoculum was aspriated, cells were washed with MEM and overlaid with Dulbecco's Modified Eagle Medium (DMEM), containing 4% Sephadex G-75 beads (Pharmacia). The plates were incubated for 2 days at 37°C, the overlay was aspirated and plates were stained with 0.5% crystal violet/80% methanol/PBS, washed and plaques enumerated.

ELISA Procedure:

The ELISA was a modification of a previously described procedure (Sabara, M. et al., J Virol (1985) 53:58-66). All incubations were performed at room temperature (20°C) for 1 hr unless stated otherwise. Polystyrene, 96-well Immunolon 2 plates (Dynatech Labs Inc., Alexandria, VA) were sensitized for each of the assay systems and used as follows

For the detection of protein specific antibody, plates were coated overnight with the respective protein (5 picomoles/well) diluted in 0.05M carbonate bicarbonate buffer at pH 9.6. Unabsorbed protein was removed by extensive washing with distilled water. The uncoated sites on the plate were blocked by overnight treatment with 3% horse serum in 0.01M PAS and then washed with double distilled H_2O. The plated antigen was overlaid

with 75 µl of mouse antiserum/well in 0.01M PAS containing 1% horse serum and 0.05% Tween 20. Incubation was carried out for 2 hr at room temperature after which time the unbound antibody was removed by washing with 0.01M PAS containing 0.05% Tween 20 (PBST). A 1/5000 dilution (in PBST plus 1% horse serum) of biotinylated-goat anti-mouse serum (Zymed Laboratories Inc., San Francisco, CA) was then added per well and incubated for one hr at room temperature. After washing in PBST, plates were incubated for one hr with 75 µl of streptavidin horseradish peroxidase conjugate diluted in PBST and 1% horse serum. After washing with PBST, the substrate (2.2'-Azino-di-[3 ethyl-benzthiazoline sulfonate], ABTS, Boehringer-Mannheim) was added. The color development was stopped after 10 min by the addition of 10% SDS. The optical density of the wells was determined at 405 nm by an ELISA reader (BioRad Laboratories, Richmond, CA). Titers were expressed as a reciprocal of the highest dilution with an OD of >2 SD over mean background levels.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE):

Viral proteins were separated by SDS-PAGE under both reducing and non-reducing conditions according to the procedure described by Laemmli (Laemmli, U.K. Nature (1970) 227:680-685). Virus samples were resuspended in electrophoresis sample buffer (0.337M Tris pH 6.8, 6% SDS, 30% glycerol, 0.03% bromophenol blue) for running under nonreducing conditions and included 3.75% mercapto-ethanol (BME) for reducing conditions. The samples were boiled for 5 to 10 min and analyzed following electrophoresis on a 10% polyacrylamide resolving gel with a 3% stacking gel.

Western Blotting of Rotavirus Proteins:

Protein-specific antibodies were detected by the Western blotting technique described by Towbin et al. (Towbin, H. et al., Proc Natl Acad Sci USA (1979) 76:4350-4354). Viral proteins separated on a 10% polyacrylamide gel, were transferred to nitrocellulose paper (0.45 μm) (BioRad Laboratories) by electroblotting at 100 volts for one hr in a buffer containing 20 mM Tris-190 mM glycine-20% methanol. Replica nitrocellulose strips were stained with amido black to determine the efficiency of protein transfer.

After transfer, reaction of viral protein with serum samples was determined as described previously (Braun, D.K. et al., J Virol (1983) 46:103-112). Non-specific reactions were blocked with 3% bovine serum albumin (BSA) in 0.01M TBS. After washing with TBST, the reaction was developed with protein A gold (BioRad Laboratories) for one hr. Following development, the protein bands were intensified by silver enhancement (Janssen Biotech, N.V., Belgium).

Example 1

Isolation of Viral Proteins

A. Isolation of Native VP6

The VP6 viral protein was isolated from the purified virus suspension (described above) by successive degradation of purified virus with EDTA and either $CaCl_2$ or LiCl, as follows. Outer capsid proteins were removed by incubating virus (3 mg/ml) in 50 mM EDTA, 0.01M Tris-HCl, pH 7.4 at 4°C for 30 min. Subviral particles were recovered by ultracentrifugation (100,000 xg, 2-3 hr, 4°C) and resuspended in 0.01M Tris-HCl, pH 7.4 or 0.01M sodium borate, pH 9.0. They were then treated with

either 1.5M $CaCl_2$ with 0.01M Tris-HCl, pH 7.4 at 20°C for 20-30 min, or were frozen in 2M LiCl, 0.01M sodium borate, pH 9.0 at -70°C for 4 days. Cores and undegraded particles were separated from solubilized protein by ultracentrifugation. EDTA and salts were removed by extensive dialysis at 4°C against 0.01M Tris-HCl, pH 7.4, unless otherwise indicated. The purity of the samples was examined by polyacrylamide gel electrophoresis (PAGE) as described above.

B. Isolation of Native VP4

The limited number of copies of VP4 protein per virus particle makes the purification of large amounts of this protein difficult. However, VP4 is found in the supernatant obtained after ultracentrifugation following treatment of the subviral particles with 1.5M $CaCl_2$ or 2M LiCl treatment of intact virus particles, as described in the isolation of native VP6. VP4 can also be purified from this pellet by e.g. HPLC, affinity chromatography, ion-exchange chromatography, etc.

C. Isolation of Native VP7

As with VP4, VP7 is also found in the supernatants described above but in larger amounts. See, Ready, K.F.M., et al. Virology (1988) 167:269-273. VP7 can be further purified using HPLC, affinity chromatography, ion-exchange chromatography, etc.

Example 2

Production of Recombinant Viral Protein

A. Production of Recombinant VP6

The construction of recombinant Autographa californica nuclear polyhedrosis virus (AcNPV) containing gene 6 from bovine rotavirus (BRV) and assembly of VP6

particles following infection of spodoptera frugiperda (SF9) cells has been described previously (Redmond, M.J. et al., Mol Immunol, In Press. Briefly, genomic RNA extracted from purified bovine rotavirus strain C486 was used to produce cDNA. The cDNA was ligated into the Pst I site of pBR322 and used to transform E. coli strain DH1. The resulting colonies were probed with radiolabeled cDNA prepared from purified genomic RNA segment 6 as template.

Clone pR6-42 which contained a complete copy of the gene 6 RNA, was partially digested with Aha III which removed seven 5' noncoding nucleotides as well as the oligo-dC tails added during cDNA cloning. A Bam HI linker was then added.

The 3' oligo-dC tail and noncoding region were removed by digestion with Acc I which removes 56 noncoding nucleotides from the VP6 gene. A Bam HI linker was then added. The gene 6 cDNA was then ligated into the Bam HI site of the baculovirus transfer vector pAc373. This vector was designated pAC373BRV6 (ATCC no. 40362). Integration of the rotavirus gene into the genome of A. californica was then carried out by homologous recombination in S. frugidperda (SF9) cells as outlined by Summers, M.D. and Smith, G.E., Texas Agricultural Station Bulletin 1555:26-27. Recombinants were identified by plaque hybridization, as described above, using radiolabeled cDNA prepared from purified genomic RNA segment 6. Recombinants were plaque purified and analyzed for expression of recombinant gene 6 produced proteins by SDS-PAGE analysis and Western blotting using the methods described above.

The recombinant virus containing gene 6 was used to infect SF9 cells. Following incubation for 72 hr at 27C, the cells were lysed in a 2 ml $NaHCO^3$ buffer (pH

7.5) containing 0.05% triton X-100 and 0.2 trypsin inhibitor units per ml. Cellular debris was removed by centrifugation at 1500g. The supernatant was dialyzed against 0.1M glycine buffer (pH 3.0) for 24 hr. This dissociates VP6 aggregates into monomers. The dialysis solution was exchanged for 0.01M citrate buffer (pH 4.0) and dialysis continued for 24 hr, during which time spheres of VP6 formed. This dialysis buffer was exchanged for 0.01M Tris-HCl (pH 7.4) + 1 mM sodium azide. Dialysis was then continued overnight at 4°C. Nonaggregated material was removed by ultracentrifugation using 300,000 dalton molecular weight cutoff filters. The quality of the VP6 spheres produced by this method was determined by electromicroscopy and purity confirmed by SDS-PAGE.

B. Production of Recombinant VP4

VP4 was produced recombinantly as described for VP6 except that site directed mutagenesis was used to insert a Bam HI restriction site immediately upstream of the translational initiation codon of the cDNA including gene 4 (the gene encoding VP4), resulting in the deletion of nine nucleotides normally present preceding the VP4 initiation codon. The 3' end of the gene was left unmodified.

The modified gene 4 was ligated into transfer vector pVL941. **[DEPOSIT NUMBER]** Integration of gene 4 into the A. californica genome was carried out as above to render the expression vector BVLVP4.

Crude detergent lysates of the VP4-baculovirus infected SF9 cells were used as starting material in the production of viral particles as described below. The interaction between VP4 and VP6 is analogous to an affinity purification step. Thus, the VP6-VP4 complex

can be separated from unbound VP4 and other cellular proteins by ultracentrifugation over sucrose gradients.

C. Production of Recombinant VP7

cDNA including a full length copy of the gene for VP7 (gene 8) was cloned and identified as described above. A clone (clone pr8-G) which contained the full length cDNA copy of the VP7 gene was digested with Aha III and Sph I which removed 7 and 27 nucleotides, respectively, from the 5' and 3' ends of the gene. Bam HI linkers were then added to both ends of the DNA.

Gene 8 was ligated into the Bam HI site of pACYM1 and integration of gene 8 into the genome of A. californica proceeded as described for VP6 to yield expression vector BYVP7. Recombinants were identified, plaque purified and assayed for expression as described above.

VP7-baculovirus infected SF9 cells secrete VP7 directly into the growth medium. VP7 is essentially affinity purified therefrom by the formation of double shelled (VP6-VP7) particles, in the presence of 50mM $CaCl_2$. This separates the VP7 from the other components present in the growth medium. The final purification step is ultracentrifugation over sucrose gradients.

Example 3

Assembly of Viral Particles

VP7 and VP4 protein concentrations in growth media and infected cell lysates were estimated on SDS-PGE gels stained with Coomassie Blue, and western blots probed with VP7 and VP4 specific antisera. A standard of native BRV was used at a predetermined concentration. The concentrations of proteins used give the following ratios to VP6

VP6-VP4. 1:10 w/w

VP6-VP7. 1:10 w/w

The starting material for all particle preparations was 10 μg of VP6. To prepare VP6-VP4 particles, 100 μg of VP4 protein in a detergent lysate of SF9 cells (described in Example 2B) dialyzed in 10 mM Tris + 50 mM $CaCl_2$ pH 8 overnight at 4°C was mixed for 2 hr. at 37° with VP6. If used as a vaccine, the preparation was centrifuged at 100,000 g. for 2 hr. through a 40% sucrose gradient.

To prepare VP6-4-7 particles, the VP6-4 particles prepared above were added to 100 μg of VP7. The VP7 preparation consisted of media from cell culture that had been dialyzed overnight into 0.1M Tris-HCl pH 8.0 containing 50 mM $CaCl_2$.

The VP6-4+7 mixture was then dialyzed for 3-4 days against 0.1M Tris-HCl pH 8.0 containing 50 mM $CaCl_2$. The particles were purified over sucrose as described previously.

To prepare VP6-VP7, 10 μg of VP6 spheres were mixed with 100 μg of VP7 contained in a preparation obtained by dialyzing tissue culture growth medium, from VP7-baculovirus infected SF9 cells, against 0.1m Tris-HCl pH 8.0 containing 50 mM $CaCl_2$. Dialysis was continued for 3-4 days against the same buffer after the addition of VP6. VP6-7 particles, when required, were purified by ultracentrifugation. In order to produce VP6-7-4 particles, cell lysates containing VP4 equivalent to 100 μg of purified virus were mixed for 2 hrs. at 37°C, with VP6-7, then ultracentrifuged through 40% Sucrose for 2 hrs. at 100,000 G. After this time, particles were purified by centrifugation as described above.

Example 4
Protective Capacity of the Assembled Viral Proteins

Vaccine compositions used in this example were as follows: For the primary immunizing dose, each immunogen (Table 1) was emulsified with Freund's complete adjuvant. For the second and third immunizations, each immunogen was emulsified with Freund's incomplete adjuvant. Equal volumes of the immunogen and the adjuvant were used.

Thirteen groups of mice were immunized intramuscularly with the preparations outlined in Table 1. Each mouse was immunized three times before and after breeding. The first immunization was given when the mice were seven weeks old and was followed by the second and third vaccinations at two week intervals. Litters were born when the mice were 12 to 14 weeks old.

Following birth, the mouse pups were allowed to suckle their dams and were challenged at 7 days of age with one of four rotavirus isolates. These isolates were bovine rotavirus strain C486 (serotype 6), simian rotavirus strain SA11 (serotype 3), human rotavirus strain DS1 (serotype 1) and Wa (serotype 2). The SA11 isolate was obtained from Dr. H. Malherbe (San Antonio, Texas) and the strain Wa and DS1 isolates were obtained from Dr. H. Greenberg (Stanford University, California). The strain C486 which was a local isolate adapted to grow in MA104 cells (Babiuk, L.A. et al., J Clin Microbiol (1977) 6:610-617). These viruses were grown in MA104 cells, harvested and concentrated for challenge by the method described previously (Ijaz et al., Antiviral Res (1987) 8:283-298. The challenge dose for each isolate was approximately 10^4 PFU/mouse suspended in MEM in 100 μl volume. For challenge, the virus preparations were

administered by intubation of the stomach with a soft flexible plastic feeding tube. Trypan blue dye (GIBCO) was used as a marker to assess the accuracy of intubation.

The appearance of diarrhea was scored clinically up to 72 hr post-challenge using clinical scores as follows:

(-) no sign of diarrhea in live mice, or on necropsy;

(+) no external signs of diarrhea but semi-liquid colon contents at autopsy;

(++) fluid was apparent on palpation of the abdomen and the colon was filled with liquid feces and gas;

(+++) the external anal region was soiled with feces and intestinal fluid was present on palpation and;

(++++) liquid feces present around the anal region and on palpation of the abdomen intestinal fluid was present and oozed from the anus, severe dehydration, internal liquid content in colon and caecum and distention due to accumulation of gas.

Table 1
HOMOLOGOUS PROTECTION OF NEONATAL MICE SUCKLING ON DAMS IMMUNIZED WITH RECOMBINANT ROTAVIRUS PROTEINS

Group	Antigen	Serum Titre	Clinical Score Wa	DS-1	SA-11	BRV	PRN
1	Sentinel	-	++++	++++	++++	++++	
2	Placebo	-	++++	++++	++++	++++	<40
3	BRV	1,540,830	0	0	0	0	>1,280
4	VP6	5,235	++	++	++	++	<40
5	VP7	6,105	ND	ND	ND	+	<40
6	VP4	53,850	0	0	+	0	780
7	VP6-VP4	31,150	0	0	+	0	1,000
8	VP6-VP7	252,520	0	0	++	0	<40
9	VP6-VP4 plus VP6-VP7 (mixture)	64,805	0	0	+	0	740
10	VP6-VP4-VP7	35,235	ND	ND	ND	0	225
11	VP6-VP7-VP4	69,685	0	0	+	0	230

Table 1 (cont'd)

Group	Antigen	Serum Titre	Clinical Score				
			Wa	DS-1	SA-11	BRV	PRN
12	VP6 (100)	53,625	ND	ND	ND	++	<40
13	VP6 (10)	29,960	ND	ND	ND	++	<40
14	VP7 (100)	4,110	ND	ND	ND	+	<40
15	VP7 (10)	27,610	ND	ND	ND	++	<40
16	VP4 (100)	22,550	ND	ND	ND	0	600
17	VP4 (10)	35,150	0	0	+	0	540
18	VP6 + VP4	59,080	ND	ND	ND	0	>1280
19	VP6 + VP7	145,820	ND	ND	ND	+	<40
20	VP6 + VP7 + VP4	44,620	ND	ND	ND	0	460

Table 1 (cont'd)

Group	Antigen	Serum Titre	Clinical Score Wa	DS-1	SA-11	BRV	PRN
21	VP6-VP7 plus insect cell proteins	544,447	ND	ND	ND	+	<40

Group 3 is bovine rotavirus antigen and was given at a dose of 50 μg/mouse.
Groups 4-6 are partially purified viral proteins. 10 μg/mouse of these proteins were administered.
Groups 7-11 and 21 are assembled particles. Approximately 10 μg of VP6 and VP7 were administered per mouse and approximately 3.2 μg of VP4 administered per mouse.
Groups 12-17 are partially purified viral proteins and the μg/mouse administered is in parentheses.
Groups 18-20 are mixed, crude lysates. Approximately 10 μg/mouse of the mixed lysate was administered and 90 μg/mouse of the insect cell protein (Group 21).
ND = Not Done
PRN = 50% plaque reduction nutralization titers.

As can be seen, vaccination with the viral particles, VP4 alone, and mixed crude cell lysates, provided protection to challenged neonatal mice.

Thus, viral particle assemblies, vaccine compositions containing these assemblies, and methods of treating and preventing rotaviral disease in a vertebrate subject are disclosed. Although preferred embodiments of the subject invention have been described in some detail, it is understood that obvious variations can be made without departing from the spirit and the scope of the intention as defined by the appended claims.

Claims

1. A viral particle assembly capable of eliciting an immunological response in a vertebrate subject, said viral particle assembly comprising:

(a) an inner capsid protein substantially homologous and functionally equivalent to VP6; and

(b) one or more outer capsid proteins selected from the group consisting of (i) a protein substantially homologous and functionally equivalent to VP4, or a functional fragment thereof, and (ii) a protein substantially homologous and functionally equivalent to VP7.

2. The viral particle assembly of claim 1 wherein (b) comprises a protein substantially homologous and functionally equivalent to VP4, or a functional fragment thereof.

3. The viral particle assembly of claim 1 wherein (b) comprises a protein substantially homologous and functionally equivalent to VP7, or a functional fragment thereof.

4. The viral particle assembly of claim 1 wherein (b) comprises a protein substantially homologous and functionally equivalent to VP4, or a functional fragment thereof, and a protein substantially homologous and functionally equivalent to VP7, or a functional fragment thereof.

5. The viral particle assembly of claim 1 wherein (a) comprises VP6 and (b) comprises VP4.

6. The viral particle assembly of claim 1 wherein (a) comprises VP6 and (b) comprises VP7.

7. The viral particle assembly of claim 1 wherein (a) comprises VP6 and (b) comprises VP4 and VP7.

8. The viral particle assembly of claim 1 wherein said (a) and (b) proteins are recombinantly produced.

9. A viral particle assembly capable of eliciting an immunological response in a vertebrate subject, said viral particle assembly comprising VP6 assembled with VP4 and VP7.

10. A vaccine composition comprising a pharmaceutically acceptable vehicle and the viral particle assembly of claim 1.

11. A vaccine composition comprising a pharmaceutically acceptable vehicle and the viral particle assembly of claim 9.

12. The vaccine composition of claim 10 further comprising one or more unassembled proteins selected from the group consisting of VP6, VP4 and VP7.

13. The vaccine composition of claim 11 further comprising one or more unassembled proteins selected from the group consisting of VP6, VP4 and VP7.

14. The vaccine composition of claim 12 wherein said unassembled protein is provided in a cell lysate.

15. The vaccine composition of claim 13 wherein said unassembled protein is provided in a cell lysate.

16. The vaccine composition of claim 10 further comprising an adjuvant.

17. The vaccine composition of claim 11 further comprising an adjuvant.

18. A method of treating or preventing rotaviral disease in a vertebrate subject comprising administering to said subject a therapeutically effective amount of a vaccine composition according to claim 10.

19. A method of treating or preventing rotaviral disease in a vertebrate subject comprising administering to said subject a therapeutically effective amount of a vaccine composition according to claim 11.

20. A method of treating or preventing rotaviral disease in a vertebrate subject comprising administering to said subject a therapeutically effective amount of a vaccine composition according to claim 12.

21. A method of treating or preventing rotaviral disease in a vertebrate subject comprising administering to said subject a therapeutically effective amount of a vaccine composition according to claim 13.

Sequence Range: 1 to 1356

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                10                  20                  30                  40
                 *                   *                   *                   *
GG CTT TTA AAC GAA GTC TTC AAC ATG GAT GTC CTG TAC TCC TTG
CC GAA AAT TTG CTT CAG AAG TTG TAC CTA CAG GAC ATG AGG AAC
                                    Met Asp Val Leu Tyr Ser Leu>

        50                  60                  70                  80
         *                   *                   *                   *
TCA AAA ACT CTT AAA GAT GCT AGA GAC AAA ATT GTC GAA GGC ACA
AGT TTT TGA GAA TTT CTA CGA TCT CTG TTT TAA CAG CTT CCG TGT
Ser Lys Thr Leu Lys Asp Ala Arg Asp Lys Ile Val Glu Gly Thr>

90                  100                 110                 120                 130
 *                   *                   *                   *                   *
TTA TAC TCC AAT GTA AGT GAT CTA ATT CAA CAA TTT AAT CAA ATG
AAT ATG AGG TTA CAT TCA CTA GAT TAA GTT GTT AAA TTA GTT TAC
Leu Tyr Ser Asn Val Ser Asp Leu Ile Gln Gln Phe Asn Gln Met>

        140                 150                 160                 170
         *                   *                   *                   *
ATA ATT ACT ATG AAT GGA AAT GAG TTC CAA ACT GGA GGA ATT GGT
TAT TAA TGA TAC TTA CCT TTA CTC AAG GTT TGA CCT CCT TAA CCA
Ile Ile Thr Met Asn Gly Asn Glu Phe Gln Thr Gly Gly Ile Gly>

180                 190                 200                 210                 220
 *                   *                   *                   *                   *
AAT CTA CCG ATT AGA AAT TGG AAT TTT GAT TTT GGA TTA CTT GGA
TTA GAT GGC TAA TCT TTA ACC TTA AAA CTA AAA CCT AAT GAA CCT
Asn Leu Pro Ile Arg Asn Trp Asn Phe Asp Phe Gly Leu Leu Gly>
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Figure 1 (Sheet 1 of 6)

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                230                 240                 250                 260
                 *                   *                   *                   *
ACA ACT CTA CTA AAT TTA GAT GCT AAC TAC GTC GAA ACG GCC CGC
TGT TGA GAT TTA AAT CTA CGA TTG ATG CAG CTT TGC CGG GCG
Thr Thr Leu Leu Asn Leu Asp Ala Asn Tyr Val Glu Thr Ala Arg>

270                 280                 290                 300                 310
 *                   *                   *                   *                   *
AAT ACA AAT GAT TAT TTT GTA GAT TTT GTA GAT AAT GTA TGT ATG
TTA TGT TTA CTA ATA AAA CAT CTA AAA CAT CTA TTA CAT ACA TAC
Asn Thr Asn Asp Tyr Phe Val Asp Phe Val Asp Asn Val Cys Met>

      320                 330                 340                 350
       *                   *                   *                   *
GAC GAA ATG GTT AGA GAA TCA CAA AGA AAT GGA ATT GCA CCA CAA
CTG CTT TAC CAA TCT CTT AGT GTT TCT TTA CCT TAA CGT GGT GTT
Asp Glu Met Val Arg Glu Ser Gln Arg Asn Gly Ile Ala Pro Gln>

360                 370                 380                 390                 400
 *                   *                   *                   *                   *
TCA GAT TCA CTT ATA AAG TTA TCA GGC ATT AAA TTT AAA AGA ATA
AGT CTA AGT GAA TAT TTC AAT AGT CCG TAA TTT AAA TTT TCT TAT
Ser Asp Ser Leu Ile Lys Leu Ser Gly Ile Lys Phe Lys Arg Ile>

      410                 420                 430                 440
       *                   *                   *                   *
AAT TTT GAC AAT TCA TCA GAA TAC ATA GAG AAC TGG AAT TTG CCA
TTA AAA CTG TTA AGT AGT CTT ATG TAT CTC TTG ACC TTA AAC GGT
Asn Phe Asp Asn Ser Ser Glu Tyr Ile Glu Asn Trp Asn Leu Pro>
```

Figure 1 (Sheet 2 of 6)

```
450          460          470          480          490
 *            *            *            *            *
 AAT AGA AGA CAA AGA ACG GGT TTT ACA TTT CAT AAA CCA AAC ATT
 TTA TCT TCT GTT TCT TGC CCA AAA TGT AAA GTA TTT GGT TTG TAA
 Asn Arg Arg Gln Arg Thr Gly Phe Thr Phe His Lys Pro Asn Ile>

      500          510          520          530
       *            *            *            *
 TTC CCT TAT TCA GCT TCA TTC ACG TTG AAC AGA TCA CAA CCT TCT
 AAG GGA ATA AGT CGA AGT AAG TGC AAC TTG TCT AGT GTT GGA AGA
 Phe Pro Tyr Ser Ala Ser Phe Thr Leu Asn Arg Ser Gln Pro Ser>

540          550          560          570          580
 *            *            *            *            *
 CAT GAT AAC TTG ATG GGT ACG ATG TGG CTC AAT GCG GGA TCA GAA
 GTA CTA TTG AAC TAC CCA TGC TAC ACC GAG TTA CGC CCT AGT CTT
 His Asp Asn Leu Met Gly Thr Met Trp Leu Asn Ala Gly Ser Glu>

      590          600          610          620
       *            *            *            *
 ATT CAG GTC GCT GGA TTC GAC TAC TCA TGT GCA ATA AAC GCG CCA
 TAA GTC CAG CGA CCT AAG CTG ATG AGT ACA CGT TAT TTG CGC GGT
 Ile Gln Val Ala Gly Phe Asp Tyr Ser Cys Ala Ile Asn Ala Pro>

630          640          650          660          670
 *            *            *            *            *
 GCT AAT ACG CAA CAA TTT GAG CAT ATT GTA CAG CTT CGA AGG GTG
 CGA TTA TGC GTT GTT AAA CTC GTA TAA CAT GTC GAA GCT TCC CAC
 Ala Asn Thr Gln Gln Phe Glu His Ile Val Gln Leu Arg Arg Val>
```

Figure 1 (Sheet 3 of 6)

```
        680                 690                 700                 710
         *                   *                   *                   *
TTG ACT ACA GCT ACA ATA ACT CTT TTA CCA GAT GCA GAA AGA TTT
AAC TGA TGT CGA TGT TAT TGA GAA AAT GGT CTA CGT CTT TCT AAA
Leu Thr Thr Ala Thr Ile Thr Leu Leu Pro Asp Ala Glu Arg Phe>

720                 730                 740                 750                 760
 *                   *                   *                   *                   *
AGT TTT CCA AGA GTG ATT ACT TCA GCT GAC GGA GCG ACT ACA TGG
TCA AAA GGT TCT CAC TAA TGA AGT CGA CTG CCT CGC TGA TGT ACC
Ser Phe Pro Arg Val Ile Thr Ser Ala Asp Gly Ala Thr Thr Trp>

        770                 780                 790                 800
         *                   *                   *                   *
TAC TTC AAT CCA GTG ATT CTT AGA CCA AAT AAC GTT GAA ATA GAG
ATG AAG TTA GGT CAC TAA GAA TCT GGT TTA TTG CAA CTT TAT CTC
Tyr Phe Asn Pro Val Ile Leu Arg Pro Asn Asn Val Glu Ile Glu>

810                 820                 830                 840                 850
 *                   *                   *                   *                   *
TTT CTA CTA AAC GGG CAG ATA ATA AAT ACT TAC CAA GCA AGA TTT
AAA GAT GAT TTG CCC GTC TAT TAT TTA TGA ATG GTT CGT TCT AAA
Phe Leu Leu Asn Gly Gln Ile Ile Asn Thr Tyr Gln Ala Arg Phe>

        860                 870                 880                 890
         *                   *                   *                   *
GGA ACC ATC ATA GCT AGA AAT TTT GAT ACA ATT AGA TTG TCA TTT
CCT TGG TAG TAT CGA TCT TTA AAA CTA TGT TAA TCT AAC AGT AAA
Gly Thr Ile Ile Ala Arg Asn Phe Asp Thr Ile Arg Leu Ser Phe>
```

Figure 1 (Sheet 4 of 6)

```
900          910          920          930          940
 *            *            *            *            *
 CAG TTG ATG AGA CCA CCA AAT ATG ACA CCA GCG GTA GCG GCG TTA
 GTC AAC TAC TCT GGT GGT TTA TAC TGT GGT CGC CAT CGC CGC AAT
 Gln Leu Met Arg Pro Pro Asn Met Thr Pro Ala Val Ala Ala Leu>

      950          960          970          980
       *            *            *            *
 TTT CCA AAT GCG CAG CCA TTT GAA CAT CAC GCA ACA GTA GGA CTC
 AAA GGT TTA CGC GTC GGT AAA CTT GTA GTG CGT TGT CAT CCT GAG
 Phe Pro Asn Ala Gln Pro Phe Glu His His Ala Thr Val Gly Leu>

990          1000         1010         1020         1030
 *            *            *            *            *
 ACG CTT AGA ATT GAA TCT GCA GTT TGT GAA TCA GTA CTT GCC GAC
 TGC GAA TCT TAA CTT AGA CGT CAA ACA CTT AGT CAT GAA CGG CTG
 Thr Leu Arg Ile Glu Ser Ala Val Cys Glu Ser Val Leu Ala Asp>

     1040         1050         1060         1070
      *            *            *            *
 GCA AGC GAA ACA ATG CTA GCA AAT GTG ACA TCT GTT AGA CAA GAA
 CGT TCG CTT TGT TAC GAT CGT TTA CAC TGT AGA CAA TCT GTT CTT
 Ala Ser Glu Thr Met Leu Ala Asn Val Thr Ser Val Arg Gln Glu>

1080         1090         1100         1110         1120
 *            *            *            *            *
 TAC GCG ATA CCA GTT GGA CCA GTT TTT CCA CCA GGT ATG AAT TGG
 ATG CGC TAT GGT CAA CCT GGT CAA AAA GGT GGT CCA TAC TTA ACC
 Tyr Ala Ile Pro Val Gly Pro Val Phe Pro Pro Gly Met Asn Trp>
```

Figure 1 (Sheet 5 of 6)

```
                                                                    1130
                                                                     *
ACT GAT TTG ATC ACT AAC TAT TCA CCA TCT AGA GAG GAT AAC TTG
TGA CTA AAC TAG TGA TTG ATA AGT GGT AGA TCT CTC CTA TTG AAC
Thr Asp Leu Ile Thr Asn Tyr Ser Pro Ser Arg Glu Asp Asn Leu>

1170
 *
CAG CGT GTA TTT ACA GTG GCT TCC ATT AGA AGC ATG CTT GTC AAA
GTC GCA CAT AAA TGT CAC CGA AGG TAA TCT TCG TAC GAA CAG TTT
Gln Arg Val Phe Thr Val Ala Ser Ile Arg Ser Met Leu Val Lys>

TGA GGA CCA AGC TAA CCA CTT GGT ATC CGA CTT TGG TGA GTA TGT
ACT CCT GGT TCG ATT GGT GAA CCA TAG GCT GAA ACC ACT CAT ACA

1260
 *
AGC TAC GTC AAG CTG TTT GAA CTC TGT AAG TAA GGA TGC GTC TAC
TCG ATG CAG TTC GAC AAA CTT GAG ACA TTC ATT CCT ACG CAG ATG

GTA TTC GCT ACA CAG AGT AAT CAC TCA GAT GGC GTA GTG AGA GGA
CAT AAG CGA TGT GTC TCA TTA GTG AGT CTA CCG CAT CAC TCT CCT

1350
 *
TGT GAC C
ACA CTG G
```

Figure 1 (Sheet 6 of 6)

Sequence Range: 1 to 451

```
                        10          20          30          40          50
                         *           *           *           *           *
Translatio  LLNEV FNMDV LYSLS KTLKD ARDKI VEGTL YSNVS DLIQQ FNQMI ITMNG

ROBMCP        15    30    45    60    75    90   105   120   135   150
[ 2164 ]    LLNEV FNMDV LYSLS KTLKD ARDKI VEGTL YSNVS DLIQQ FNQMI ITMNG>
            ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO1HVP6       15    30    45    60    75    90   105   120   135   150
[ 2061 ]    LLNEV FNMDV LYSLS KTLKD ARDKI VEGTL YSNVS DLIQQ FNQMI ITMNG>
            ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO1VVP6H2     15    30    45    60    75    90   105   120   135   150
[ 1997 ]    LLNEV FNMDV LYSLS KTLKD ARDKI VEGTL YSNVS DLIQQ FNQMv ITMNG>
            ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO1VVP6FI     15    30    45    60    75    90   105   120   135   150
[ 1974 ]    LLNEV FNMeV LYSiS KTLKD ARDKI VEGTL YSNVS DiIQQ FNQiI vTMNG>
            ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO1PVP6       15    30    45    60    75    90   105   120   135   150
[ 1949 ]    LLNEV FNMeV LYSLS KTLKD ARDKI VEGTL YSNVS DLIQQ FNQMI vTMNG>
            ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO2SEG6       15    30    45    60    75    90   105   120   135   150
[ 1939 ]    L*NEV FdMeV LYSLS KTLKD ARDKI VEGTL YSNVS DLIQQ FNQMI vTMNG>
            ^v^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

PRVVP6          25    40    55    70    85   100   115   130   145
[  904 ]      FtMDV LfSia KTvsD lkkKv VvGTi YtNVe DiIQQ tNelI rTlNG>
              ^ ^^^ ^^^^  ^^^ ^ v^ ^^ ^v^^^ ^ ^^  ^^^^^ v^^^^ v^^^^
```

Figure 2 (Sheet 1 of 10)

```
                                60          70          80          90         100
                                 *           *           *           *           *
Translatio  NEFQT GGIGN LPIRN WNFDF GLLGT TLLNL DANYV ETARN TNDYF VDFVD

ROBMCP      165  180  195  210  225  240  255  270  285  300
[ 2164 ]    NEFQT GGIGN LPIRN WNFDF GLLGT TLLNL DANYV ETARN TiDYF VDFVD>
            ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO1HVP6     165  180  195  210  225  240  255  270  285  300
[ 2061 ]    NEFQT GGIGN LPIRN WNFDF GLLGT TLLNL DANYV ETARN TiDYF VDFVD>
            ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO1VVP6H2   165  180  195  210  225  240  255  270  285  300
[ 1997 ]    NEFQT GGIGN LPIRN WNFDF GLLGT TLLNL DANYV ETARN TiDYF VDFVD>
            ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO1VVP6FI   165  180  195  210  225  240  255  270  285  300
[ 1974 ]    NEFQT GGIGt LPIRN WtFDF GLLGT TLLNL DANYV ETARt TieYF iDFiD>
            ^^^^^ ^^^^  ^^^^^ ^ ^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^  ^V^^^ ^^^^^

RO1PVP6     165  180  195  210  225  240  255  270  285  300
[ 1949 ]    NdFQT GGIGN LPvRN WtFDF GLLGT TLLNL DANYV ETARt TieYF iDFiD>
            ^^^^^ ^^^^^ ^^^^^ ^ ^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^  ^V^^^ ^^^^^

RO2SEG6     165  180  195  210  225  240  255  270  285  300
[ 1939 ]    NdFQT GGIGN LPvRN WtFDF GLLGT TLLNL DANYV EnARt iieYF iDFiD>
            ^^^^^ ^^^^^ ^^^^^ ^ ^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^^  V^^^ ^^^^^

PRVVP6      160  175  190  205  220  235  250  265  280  295
[  904 ]    NtFhT GGIGt qPqke WNFql pqLGT TLLNL DdNYV qatRs viDYl asFie>
            ^ ^^^ ^^^^  V^V^  ^^^^^ V^^^ ^^^^^ ^ ^^^ ^  ^  V^^^  ^^^
```

Figure 2 (Sheet 2 of 10)

```
                    110       120       130       140       150
                     *         *         *         *         *
Translatio NVCMD EMVRE SQRNG IAPQS DSLIK LSGIK FKRIN FDNSS EYIEN WNLPN
ROBMCP     315 330 345 360 375 390 405 420 435 450
[ 2164 ]   NVCMD EMVRE SQRNG IAPQS DSLIK LSGIK FKRIN FDNSS EYIEN WNLPN>
           ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^
RO1HVP6    315 330 345 360 375 390 405 420 435 450
[ 2061 ]   NVCMD EMVRE SQRNG IAPQS DSLrK LSGIK FKRIN FDNSS EYIEN WNLqN>
           ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^v^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^ ^
RO1VVP6H2  315 330 345 360 375 390 405 420 435 450
[ 1997 ]   NVCMD EMVRE SQRNG IAPQS DSLrK LSGIK FKRIN FDNSS EYIEN WNLqN>
           ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^v^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^ ^
RO1VVP6FI  315 330 345 360 375 390 405 420 435 450
[ 1974 ]   NVCMD EMtRE SQRNG IAPQS DaLrK LSGIK FKRIN FDNSS EYIEN WNLqN>
           ^^^^^ ^^ ^^ ^^^^^ ^^^^^ ^ ^v^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^ ^
RO1PVP6    315 330 345 360 375 390 405 420 435 450
[ 1949 ]   NVCMD EMaRE SQRNG IAPQS eafrK LaGIK FKRIN FDNSS EYIEN WNLqN>
           ^^^^^ ^^ ^^ ^^^^^ ^^^^^ ^ ^v^ ^ ^^^ ^^^^^ ^^^^^ ^^^^^ ^^^ ^
RO2SEG6    315 330 345 360 375 390 405 420 435 450
[ 1939 ]   NVCMD EMaRE SQRNG vAPQS eaLrK LaGIK FKRIN FDNSS EYIEN WNLqN>
           ^^^^^ ^^ ^^ ^^^^^ ^^^^^ ^ ^v^ ^ ^^^ ^^^^^ ^^^^^ ^^^^^ ^^^ ^
PRVVP6     310 325 340 355 370 385 400 415 430 445
[ 904 ]    aVCdD EiVRE asRNG mqPQS ptLIa LassK FKtIN FnNSS qsIkN ws-aq>
           ^v^ ^^^^^ ^^^ ^ ^^^ ^^ ^ ^ ^^ ^^ ^^^^^ ^v^ ^ ^
```

Figure 2 (Sheet 3 of 10)

```
RO1VVP6H2.                                    135       150   165   180   195
[   40 ]                                        K Lk-l- FKRdv Frkrt sklEN rkL>
                                                ^ ^   ^  ^^^vv ^      v^^^ ^ ^

                        160         170         180         190         200
                         *           *           *           *           *
Translatio  RRQRT GFTFH KPNIF PYSAS FTLNR SQPSH DNLMG TMWLN AGSEI QVAGF

ROBMCP        465   480   495   510   525   540   555   570   585   600
[ 2164 ]    RRQRT GFTFH KPNIF PYSAS FTLNR SQPaH DNLMG TMWLN AGSEI QVAGF>
            ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^ ^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO1HVP6       465   480   495   510   525   540   555   570   585   600
[ 2061 ]    RRQRT GFTFH KPNIF PYSAS FTLNR SQPaH DNLMG TMWLN AGSEI QVAGF>
            ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^ ^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO1VVP6H2     465   480   495   510   525   540   555   570   585   600
[ 1997 ]    RRQRk GFTFH KPNIF PYSAS FTLNR SQPaH DNLMG TMWLN AGSEI QVAGF>
            ^^^^  ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^ ^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO1VVP6FI     465   480   495   510   525   540   555   570   585   600
[ 1974 ]    RRQRT GFvFH KPNIF PYSAS FTLNR SQPlH ndLMG TMWLN AGSEI QVAGF>
            ^^^^^ ^^ ^^ ^^^^^ ^^^^^ ^^^^^ ^^^v^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO1PVP6       465   480   495   510   525   540   555   570   585   600
[ 1949 ]    RRQRT GFiFH KPNIF PYSAS FTLNR SQPmH DNLMG TMWLN AGSEI QVAGF>
            ^^^^^ ^^ ^^ ^^^^^ ^^^^^ ^^^^^ ^^^v^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO2SEG6       465   480   495   510   525   540   555   570   585   600
[ 1939 ]    RRQRT GFvFH KPNIF PYSAS FTLNR SQPmH DNLMG TMWLN AGSEI QVAGF>
            ^^^^^ ^^ ^^ ^^^^^ ^^^^^ ^^^^^ ^^^v^ ^^^^^ ^^^^^ ^^^^^ ^^^^^
```

Figure 2 (Sheet 4 of 10)

```
PRVVP6          460   475   490   505   520   535   550   565   580   595
[  904 ]        sgvRi qFmni nPmvF eYrnS yiLqR anPqy gNvMG lryyt Asntc QlAaF>
                vv^   ^ vv  ^v^^  ^ ^ ^ ^ ^ ^   ^^^^ v   ^   v ^^^ ^
                        210   220   230   240   250
                          *     *     *     *     *
Translatio      DYSCA INAPA NTQQF EHIVQ LRRVL TTATI TLLPD AERFS FPRVI TSADG

ROBMCP          615   630   645   660   675   690   705   720   735   750
[ 2164 ]        DYSCA INAPA NTQQF EHIVQ LRRVL TTATI TLLPD AERFS FPRVI TSADG>
                ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO1HVP6         615   630   645   660   675   690   705   720   735   750
[ 2061 ]        DYSCA INAPA NTQQF EHIVQ LRRVL TTATI TLLPD AERFS FPRVI nSADG>
                ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^

RO1VVP6H2       615   630   645   660   675   690   705   720   735   750
[ 1997 ]        DYSCA INAPA NTQQF EHIVQ LRRVL TTATI TLLPD AERFS FPRVI nSADG>
                ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^

RO1VVP6FI       615   630   645   660   675   690   705   720   735   750
[ 1974 ]        DYSCA INAPA NTQQF EHIVQ LRRaL TTATI TiLPD AERFS FPRVI nSADG>
                ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^ ^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^

RO1PVP6         615   630   645   660   675   690   705   720   735   750
[ 1949 ]        DYSCA INAPA NiQQF EHIVQ LRRaL TTATI TLLPD AERFS FPRVI nSADG>
                ^^^^^ ^^^^^ ^ ^^^ ^^^^^ ^^^ ^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^

RO2SEG6         615   630   645   660   675   690   705   720   735   750
[ 1939 ]        DYSCA INAPA NiQQF EHIVQ LRRaL TTATI TLLPD AERFS FPRVI nSADG>
                ^^^^^ ^^^^^ ^ ^^^ ^^^^^ ^^^ ^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^
```

Figure 2 (Sheet 5 of 10)

```
PRVVP6          610       625       640       655       670       685       700       715       730       745
[  904 ]  DstlA eNAPn NTQrF iyngr LkRpi snvlm kieag Apnin nltil pdptn>
          ^v v^ v^^^  ^^^ ^ v v   ^^^ ^   v^  ^v  ^     vv ^^
                    260       270       280       290       300
                     *         *         *         *         *
Translatio ATTWY FNPVI LRPNN VEIEF LLNGQ IINTY QARFG TIIAR NFDTI RLSFQ

ROBMCP          765       780       795       810       825       840       855       870       885       900
[ 2164 ]  ATTWY FNPVI LRPNN VEIEF LLNGQ IINTY QARFG TIIAR NFDTI RLSFQ>
          ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO1HVP6         765       780       795       810       825       840       855       870       885       900
[ 2061 ]  ATTWY FNPVI LRPNN VEvEF LLNGQ IINTY QARFG TIIAR NFDTI RLSFQ>
          ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO1VVP6H2       765       780       795       810       825       840       855       870       885       900
[ 1997 ]  tTTWY FNPVI fRPNN VEIEF LLNGQ IINnY QARFG TIIAR NFDTI RLSFQ>
          ^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^ ^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO1VVP6FI       765       780       795       810       825       840       855       870       885       900
[ 1974 ]  ATTWf FNPVI LRPNN VEvEF LLNGQ IINTY QARFG TIIAR NFDTI RLSFQ>
          ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO1PVP6         765       780       795       810       825       840       855       870       885       900
[ 1949 ]  ATTWf FNPVI LRPNN VEvEF LLNGQ IINTY QARFG TIVAR NFDTI RLSFQ>
          ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO2SEG6         765       780       795       810       825       840       855       870       885       900
[ 1939 ]  ATTWf FNPVI LRPNN VEvEF LLNGQ IINTY QARFG TIIAR NFDaI RL1FQ>
          ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^ ^ ^^v^^
```

Figure 2 (Sheet 6 of 10)

```
PRVVP6          760   775   790   805   820   835   850   865   880
[  904 ]  qTTW1 yNPdq Lmngt ftIEF ynNGQ lvdmv r-nmG vvtvR tFDsy Ritid>
          ^^^^  ^^^vv ^     ^^^  v^^^ ^^^ v   ^   ^ ^  ^^  ^^  ^ ^

                    310           320           330           340           350
                     *             *             *             *             *
Translatio      LMRPP NMTPA VAALF PNAQP FEHHA TVGLT LRIES AVCES VLADA SETML

ROBMCP          915   930   945   960   975   990  1005  1020  1035  1050
[ 2164 ]  LMRPP NMTPA VAALF PNAQP FEHHA TVGLT LRIES AVCES VLADA SETML>
          ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO1HVP6         915   930   945   960   975   990  1005  1020  1035  1050
[ 2061 ]  LMRPP NMTPt VAALF PNAQP FEHHA TVGLT LRIES AVCES VLADA SETML>
          ^^^^^ ^^^^  ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

                pp
                |-|
RO1VVP6H2       930   945   960   975   990  1005  1020  1035  1050
[ 1997 ]  LMRPq NMTPA VAALF PNApP FEHHA TVGLT LRIES AiCES VLADA SETML>
          ^^^^  ^^^^^ ^^^^^ ^^^ ^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO1VVP6FI       915   930   945   960   975   990  1005  1020  1035  1050
[ 1974 ]  LMRPP NMTPA VnALF PqAQP FqHHA TVGLT LRIdS AVCES VLADs nETML>
          ^^^^^ ^^^^^ ^ ^^^ ^ ^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^  ^^^^

RO1PVP6         915   930   945   960   975   990  1005  1020  1035  1050
[ 1949 ]  LMRPP NMTPA VdALF PqAQP FqHHA TVGLT LRIES AVCES VLADA nETlL>
          ^^^^^ ^^^^^ ^ ^^^ ^ ^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^
```

Figure 2 (Sheet 7 of 10)

```
RO2SEG6          915   930   945   960   975   990  1005  1020  1035  1050
[ 1939 ]     LMRPP NMTPA VnALF PqAQP FqHHA TVGLT LRIES AVCES VLADA nETlL>
             ^^^^^ ^^^^^ ^ ^^^ ^ ^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^

PRVVP6       895   910   925   940   955   970   985  1000  1015  1030
[  904 ]     miRPa aMTqy VqrLF PqggP ypyqA aymLT LsIld AttES VLcDs hsvdy>
             ^^^^  ^^ v ^ v^^ ^ ^ ^ ^^  vv^^ ^ ^v  ^ v^^ ^^v^    v

                     360           370           380           390           400
                     *             *             *             *             *
Translatio   ANVTS VRQEY AIPVG PVFPP GMNWT DLITN YSPSR EDNLQ RVFTV ASIRS

ROBMCP       1065  1080  1095  1110  1125  1140  1155  1170  1185  1200
[ 2164 ]     ANVTS VRQEY AIPVG PVFPP GMNWT DLITN YSPSR EDNLQ RVFTV ASIRS>
             ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO1HVP6      1065  1080  1095  1110  1125  1140  1155  1170  1185  1200
[ 2061 ]     ANVTS VRQEY AIPVG PVFPP GMNWT DLITN YSPSR EDNLQ RVFTV ASIRS>
             ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO1VVP6H2    1065  1080  1095  1110  1125  1140  1155  1170  1185  1200
[ 1997 ]     ANVTS VRQEY AvPVG PVFPP GMNWT DLITN YSPSR EDNLQ RVFTV ASIRS>
             ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO1VVP6FI    1065  1080  1095  1110  1125  1140  1155  1170  1185  1200
[ 1974 ]     ANVTa VRQEY AvPVG PVFPP GMNWT eLITN YSPSR EDNLQ RVFTV ASIRS>
             ^^^^  ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO1PVP6      1065  1080  1095  1110  1125  1140  1155  1170  1185  1200
[ 1949 ]     ANVTa VRQEY AIPVG PVFPP GMNWT eLITN YSPSR EDNLQ RVFTV ASIRS>
             ^^^^  ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^
```

Figure 2 (Sheet 8 of 10)

```
RO2SEG6      1065  1080  1095  1110  1125  1140  1155  1170  1185  1200
[ 1939 ]     ANVTa VRQEY AIPVG PVFPP GMNWT eLITN YSPSR EDNLQ RVFTV ASIRS>
             ^^^^  ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

PRVVP6       045  1060  1075  1090  1105  1120  1135  1150  1165  1180
[  904 ]     siVan wRrds AmPaG tVFqP GfpWe qtlsN Ytvaq EDNLe R11lV ASvkr>
               v^   v^ ^v ^^^ ^  ^^ ^ ^ ^v^ ^ ^   ^^^^^ ^^^v^ ^^^^

                      410           420           430           440           460
                       *             *             *             *             *
Translatio   MLVK* GPS*P LGIRL W*VCS YVKLF ELCK* GCVYV FATQS NHSDG VVRGC

ROBMCP       1215  1230  1245  1260  1275  1290  1305  1320  1335  1350
[ 2164 ]     MLVK* GPS*P LGIRL W*VCS YVKLF ELCK* GCVYV FATQS NHSDG VVRGC>
             ^^^^v ^^^v^ ^^^^^ ^v^^^ ^^^^^ ^^^^v ^^^^^ ^^^^^ ^^^^^ ^^^^^

RO1HVP6      1215  1230  1245  1260  1275  1290  1305  1320  1335
[ 2061 ]     MLiK* GPS*l LGIRt l*aCS YVKLF ELCK* GCiYt FATQS NHfnd Vkk>
             ^^^^v ^^^vv ^^^^v vv ^^ ^^^^^ ^^^^v ^^^^  ^^^^^ ^^v^  ^v^

RO1VVP6H2    1215  1230  1245  1260  1275  1290  1305  1320  1335  1350
[ 1997 ]     MLiK* rPd*q sGIRf y1aCS YiKLy rLCK* GyiYi FtTQS NctDG ikk>
             ^^^^v v^ v  v^^^^  v ^^ ^^^^^ ^^^^v ^ ^^^ ^ ^^^ ^v ^^ ^v^

RO1VVP6FI    1215  1230  1245  1260  1275  1290  1305  1320  1335
[ 1974 ]     MLiK* GPS*l LGI1v **VCS YVKL1 ELCK* ehVhV FvpQS NHSDG ikk>
             ^^^^v ^^^vv ^^^v^ vv^^^ ^^^^^ ^^^^v v^ ^ ^ ^^ ^^^^^ ^v^

RO1PVP6      1215  1230  1245  1260  1275  1290  1305  1320  1335
[ 1949 ]     MLiK* GPd*t sGIqp l1aCS YiKsF rLCK* Ghdfi FAT*S Nc1nd Vkk>
             ^^^^v ^^ v  v^^ v vv ^^ ^^^v^ ^^^^v ^vv^^ ^^^v^ ^vv^  ^v^
```

Figure 2 (Sheet 9 of 10)

```
RO2SEG6      1215  1230  1245  1260  1275  1290  1305  1320  1335  1350
[ 1939 ]     MLiK* GPd*a sGIqs *laCS YiKsF rLfK* Ghdfm FAT*S Nclnd VVRGC>
             ^^^^v ^^ v  v^^ v vv ^^ ^^^v^ ^v^v ^vv^^ ^^^v^ ^vv^  ^^^^^

PRVVP6     195
[  904 ]     Mvm>
             ^^^

Translatio  D

ROBMCP
[ 2164 ]     D>
             ^

RO2SEG6
[ 1939 ]     D>
             ^
```

Figure 2 (Sheet 10 of 10)

```
Sequence Range: 1 to 776

                    10                  20                  30                  40                  50
                    *                   *                   *                   *                   *
C486G4p     MASLI YRQLL TNSYT VELSD EIQEI GSTKT QNVTV NPGPF AQTNY ASVNW

SA11G4p         5   10  15  20  25  30  35  40  45  50
[ 3506 ]    MAaLI YRQLL TNSYT VELSD EIQEI GSTKT QNVTV NPGPF AQTNY ApVNW>
            ^^ ^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^^^

RRVVP4p         5   10  15  20  25  30  35  40  45  50
[ 3198 ]    MASLI YRQLL TNSYT VdLSD EIQEI GSTKT QNVTi N1GPF AQTgY ApVNW>
            ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^^^ ^^^ ^ ^^^

DS-1p           5   10  15  20  25  30  35  40  45  50
[ 2870 ]    MASLI YRQLL TNSYs VdLhD EIeqI GSeKT QsVTV NPGPF AQTrY ApVNW>
            ^^^^^ ^^^^^ ^^^^  ^^^ ^ ^^^^^ ^^ ^^ ^ ^^^ ^^^^^ ^^^ ^ ^^^

M37p            5   10  15  20  25  30  35  40  45  50
[ 2844 ]    MASLI YRQLL TNSYs VELSD EIntI GSeKT QNVTi NPGPF AQTNY ApVvl>
            ^^^^^ ^^^^^ ^^^^  ^^^^^ ^^  ^ ^^ ^^ ^^^^^ ^^^^^ ^^^^^ ^ ^

KUp             5   10  15  20  25  30  35  40  45  50
[ 2837 ]    MASLI YRQLL TNSYs VdLhD EIeqI GSeKT QNVTV NPGPF AQTrY ApVNW>
            ^^^^^ ^^^^^ ^^^^  ^^^ ^ ^^^^^ ^^ ^^ ^^^^^ ^^^^^ ^^^ ^ ^^^

ST3p            5   10  15  20  25  30  35  40  45  50
[ 2836 ]    MASLI YRQLL TNSYT VELSD EIntI GSeKs QNiTi NPGPF AQTNY ApVvl>
            ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^  ^ ^^ ^  ^^^^^ ^^^^^ ^^^^^ ^ ^

K8p             5   10  15  20  25  30  35  40  45  50
[ 2735 ]    MASLI YRQLL sNSYv tniSD EvnEI GtkKT tNVTV NPGPF AQTgY ApVdW>
            ^^^^^ ^^^^^ ^^^^  ^^^ ^^ ^^ ^ ^^ ^^^^^ ^^^^^ ^^^ ^ ^^^
```

Figure 3 (Sheet 1 of 17)

```
                       60          70          80          90         100
                        *           *           *           *           *
C486G4p     GPGET NDSTT VEPVL DGPYQ PTTFN PPVSY WMLLA PTNAG VVDQG TNNTN

SA11G4p        55    60    65    70    75    80    85    90    95   100
[ 3506 ]    GPGET NDSTT VEPVL DGPYQ PTTFN PPVSY WMLLA PTNAG VVveG TNNTN>
            ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^ ^^ ^^^^^

RRVVP4p        55    60    65    70    75    80    85    90    95   100
[ 3198 ]    GPGET NDSTT VEPVL DGPYQ PTsFN PPVdY WMLLA PTaAG VVveG TNNTd>
            ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^ ^^ ^^^ ^ ^^^^^ ^^ ^^ ^^ ^^ ^^^^^

DS-1p          55    60    65    70    75    80    85    90    95   100
[ 2870 ]    GhGEi NDSTT VEPVL DGPYQ PTTFk PPtdY WlLis sntnG VVyes TNNnd>
            ^ ^^  ^^^^^ ^^^^^ ^^^^^ ^^^^  ^^  ^ ^^^^      ^ ^^ ^  ^^^ ^

                                        s
                                        |
M37p           55    60    65    70    75| 80    85    90    95   100
[ 2844 ]    eswEv NDSTT iEPVL DGPYQ P-TFk PPtdY WiLLn PTdqq VVleG TNkTd>
                ^ ^^^^^ ^^^^^ ^^^^^ ^ ^^  ^^  ^ ^^^^  ^^^   ^^ ^^ ^^ ^^

KUp            55    60    65    70    75    80    85    90    95   100
[ 2837 ]    GhGEi NDSTT VEPiL DGPYQ PTTFk PltdY WiLin sntnG VVyes TNNsd>
            ^ ^^  ^^^^^ ^^^^^ ^^^^^ ^^^^  ^   ^ ^^^^      ^ ^^ ^  ^^^ ^

                                        s
                                        |
ST3p           55    60    65    70    75| 80    85    90    95   100
[ 2836 ]    eswEv NDSTT iEPVL DGPYQ P-TFk PPtdY WiLLn PTNqq VVleG TNkTd>
                ^ ^^^^^ ^^^^^ ^^^^^ ^ ^^  ^^  ^ ^^^^  ^^^   ^^ ^^ ^^ ^^
```

Figure 3 (Sheet 2 of 17)

```
K8p                55        60        65        70        75        80        85        90        95       100
[ 2735 ]           GhGEl pDST1 VqPtL DGPYQ PTlnl PvtdY WMLiA PTreG kVaeG TNtTd>
                   ^ ^^   ^^^  ^^^ ^ ^^^^^ ^^   ^  ^ ^^^^^ ^^  ^  ^ ^^ ^^ ^^

                          110       120       130       140       150
                            *         *         *         *         *
C486G4p            RWLAT ILIKP NVQQV ERTYT LFGQQ VQVTV SNDSQ TKWKF VDLSK QTQDG

SA11G4p            105       110       115       120       125       130       135       140       145       150
[ 3506 ]           RWLAT ILIeP NVQQV ERTYT LFGQQ VQVTV SNDSQ TKWKF VDLSK QTQDG>
                   ^^^^^ ^^^ ^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RRVVP4p            105       110       115       120       125       130       135       140       145       150
[ 3198 ]           RWLAT ILVeP NVtse tRsYT LFGtQ eQiTi ayaSQ TqWKF iDvvK tTQnG>
                   ^^^^^ ^^^ ^ ^^    ^ ^^ ^^^ ^ ^^^^ ^^ ^ ^^^ ^^^ ^ ^^^^

DS-1p              105       110       115       120       125       130       135       140       145
[ 2870 ]           fWtAv IaIeP hVsQV nRqYT LFGen kQfnV eNnSd -KWKF femfK gssqG>
                   ^ ^   ^ ^ ^ ^^ ^^ ^ ^^ ^^^^ ^  ^ ^^^^ ^^^^ ^^ ^ ^^

M37p               105       110       115       120       125       130       135       140       145
[ 2844 ]           iWiAl lLveP NVtnq sRqYT LFGet kQiTV eNntn -KWKF femfr knvsa>
                   ^^^  ^^^ ^ ^^    ^ ^^ ^^^^ ^^^^ ^^^^ ^^   ^^^^ ^^ ^ ^

KUp                105       110       115       120       125       130       135       140       145
[ 2837 ]           fWtAv vaIeP hViQV dRqYT vFGen kQfnV rNDSd -KWKF lemfr gssqn>
                   ^ ^   ^ ^ ^ ^^ ^^ ^^ ^^^^ ^  ^ ^^^^ ^^^^ ^^^ ^ ^   ^

ST3p               105       110       115       120       125       130       135       140       145
[ 2836 ]           iWiAl lLveP NVtnq sRqYT LFGet kQiTV eNntn -KWKF femfr ssvss>
                   ^^^  ^^^ ^ ^^    ^ ^^ ^^^^ ^^^^ ^^^^ ^^   ^^^^ ^^ ^ ^
```

Figure 3 (Sheet 3 of 17)

```
K8p                105   110   115   120   125   130   135   140   145   150
[ 2735 ]           RWfAc vLveP NVQnt qRqYv LdGQn VQlhV SNDSs TsWKF ilfiK lTpyG>
                   ^^^^  ^^^ ^ ^^^  ^^ ^  ^ ^^  ^^^ ^ ^^^^  ^ ^^^ ^ ^ ^  ^ ^

                         160   170   180   190   200
                           *     *     *     *     *
C486G4p                  NYSQH GPLLS TPKLY GVMKH GGKIY TYNGE TPNAT TGYYS TTNFD TVNMT

SA11G4p            155   160   165   170   175   180   185   190   195   200
[ 3506 ]           NYSQH GsLLS TPKLY GVMKH GGKIY TYNGE TPNAn TGYYS TTNFD TVNMT>
                   ^^^^^ ^ ^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^ ^^^^  ^^^^^ ^^^^^ ^^^^^

RRVVP4p            155   160   165   170   175   180   185   190   195   200
[ 3198 ]           sYSQy GPLqS TPKLY aVMKH nGKIY TYNGE TPNvT TkYYS TTNyD sVNMT>
                   ^^^  ^^^ ^ ^^^^^ ^^^^  ^^^^ ^^^^^ ^^^ ^ ^ ^^^ ^^^^^  ^^^^

DS-1p              150   155   160   165   170   175   180   185   190   195
[ 2870 ]           NfSnr rtLtS snrLv GmlKy GGrvw TfhGE TPrAT Tdssn Tadln nisii>
                   ^^^ ^   ^ ^   ^^   ^^^^  ^^^^  ^^^^^ ^^ ^^ ^   ^ ^^^  ^ ^

M37p               150   155   160   165   170   175   180   185   190   195
[ 2844 ]           efqhk rtLtS dtKLa GflKH ynsvw TfhGE TPhAT TdYsS TsN1s eVetv>
                   ^ ^   ^ ^   ^^  ^ ^^^   ^   ^^^^^ ^^^^^ ^ ^ ^ ^ ^^   ^

KUp                150   155   160   165   170   175   180   185   190   195
[ 2837 ]           efynr rtLtS dtKLv GilKy GGrIw TfhGE TPrAT Tdssn TaN1n disii>
                   ^ ^   ^ ^   ^^   ^^^^  ^^^^  ^^^^^ ^^ ^^ ^   ^ ^^^  ^ ^

ST3p               150   155   160   165   170   175   180   185   190   195
[ 2836 ]           efqhk rtLtS dtKLa GflKH ynsvw sfhGE TPhAT TdYsS TsN1s eVetv>
                   ^ ^   ^ ^   ^^   ^ ^^^   ^   ^^^^ ^^^^^ ^ ^ ^ ^ ^^   ^
```

Figure 3 (Sheet 4 of 17)

```
K8p                155  160  165  170  175  180  185  190  195  200
[ 2735 ]      tYtQy stLst phKLc awMKr dnrvY wYqGa TPNAs esYY1 TiNnD nsNvs>
              ^ ^   ^    ^^   ^^^  ^^^  ^ ^  ^^^^  ^^   ^ ^ ^  ^^

                        210  220  230  240  250
                         *    *    *    *
C486G4p       AVCDF YIIPL AQEAK CTEYI NNGLP PIQNT RNIVP VSIVS RNIVY TRAQP

                   205  210  215  220  225  230  235  240  245  250
SA11G4p            *    *    *    *    *    *    *    *    *    *
[ 3506 ]      AVCDF YIIPL AQEAK CTEYI NNGLP PIQNT RNIVP VSIVS RNIVY TRAQP>
              ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

                   205  210  215  220  225  230  235  240  245  250
RRVVP4p            *    *    *    *    *    *    *    *    *    *
[ 3198 ]      AfCDF YIIPr eeEst CTEYI NNGLP PIQNT RNIVP lalsa RNIis hRAQa>
              ^^^^^ ^^^^  ^^   ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^   ^^^^  ^^^

                   200  205  210  215  220  225  230  235  240  245
DS-1p              *    *    *    *    *    *    *    *    *    *
[ 2870 ]      ihseF YIIPr sQEsK CnEYI NNGLP PIQNT RNvVP lSlsS RsIqY rRAQv>
              ^^ ^^^^  ^^ ^ ^ ^^^ ^^^^^ ^^^^^ ^^^^^ ^^^ ^ ^ ^ ^  ^^^

                   200  205  210  215  220  225  230  235  240  245
M37p               *    *    *    *    *    *    *    *    *    *
[ 2844 ]      ihveF YIIPr sQEsK CvEYI NtGLP PmQNT RNIVP ValsS RsvtY qRAQv>
              ^^ ^^^^  ^^ ^ ^ ^^^ ^ ^^^ ^^^^^ ^^^^^ ^ ^ ^ ^ ^  ^^^

                   200  205  210  215  220  225  230  235  240  245
KUp                *    *    *    *    *    *    *    *    *    *
[ 2837 ]      ihseF YIIPr sQEsK CnEYI NNGLP PIQNT RNvVP lSlsS RsIqY kRAQv>
              ^^ ^^^^  ^^ ^ ^ ^^^ ^^^^^ ^^^^^ ^^^^^ ^^^ ^ ^ ^ ^  ^^^

                   200  205  210  215  220  225  230  235  240  245
ST3p               *    *    *    *    *    *    *    *    *    *
[ 2836 ]      ihveF YIIsr sQEsK CvEYI NtGLP PmQNT RNIVP ValsS RsvtY qRAQv>
              ^^ ^^^  ^^ ^ ^ ^^^ ^ ^^^ ^^^^^ ^^^^^ ^ ^ ^ ^ ^  ^^^
```

Figure 3 (Sheet 5 of 17)

```
K8p                205   210   215   220   225   230   235   240   245   250
[ 2735 ]     sdaeF YlIPq sQtAm CTqYI NNGLP PIQNT RNIVP VnItS RqIkd vRAQm>
               ^^ ^^^^   ^ ^  ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^ ^ ^ ^    ^^^

                         260         270         280         290         300
                          *           *           *           *           *
C486G4p      NQDIV VSKTS LWKEM QYNRD IVIRF KFANS IIKSG GLGYK WSEVS FKPAN

SA11G4p        255   260   265   270   275   280   285   290   295   300
[ 3506 ]     NQDIV VSKTS LWKEM QYNRD IVIRF KFANS IIKSG GLGYK WSEVS FKPAf>
               ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^

RRVVP4p        255   260   265   270   275   280   285   290   295   300
[ 3198 ]     NeDIV VSKTS LWKEM QYNRD ItIRF KFAsS IvKSG GLGYK WSEiS FKPAN>
               ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^^^ ^^^ ^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

DS-1p      250   255   260   265   270   275   280   285   290   295
[ 2870 ]     NeDIt iSKTS LWKEM QYNRD IiIRF KFgNS vIKlG GLGYK WSEiS yKaAN>
               ^^^^  ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^ ^^ ^^^ ^ ^^^^^ ^^^^^ ^^ ^^

M37p       250   255   260   265   270   275   280   285   290   295
[ 2844 ]     NeDIi iSKTS LWKEM QcNRD IiIRF KFnNS IvKlG GLGYK WSEiS FKaAN>
               ^^^^^ ^^^^^ ^^^^^ ^ ^^^ ^^^^^ ^^ ^^ ^^^ ^ ^^^^^ ^^^^^ ^^ ^^

KUp        250   255   260   265   270   275   280   285   290   295
[ 2837 ]     NeDIt iSKTS LWKEM QcNRD IiIRF KFgNS IvKlG GLGYK WSEiS yKaAN>
               ^^^^  ^^^^^ ^^^^^ ^ ^^^ ^^^^^ ^^ ^^ ^^^ ^ ^^^^^ ^^^^^ ^^ ^^

ST3p       250   255   260   265   270   275   280   285   290   295
[ 2836 ]     NeDIi iSKTS LWKEM QYNRD IiIRF KFnNS IIKlG GLGYK WSEiS FKaAN>
               ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^ ^^ ^^^ ^ ^^^^^ ^^^^^ ^^ ^^
```

Figure 3 (Sheet 6 of 17)

```
K8p                255  260  265  270  275  280  285  290  295  300
[ 2735 ]      NeDIV iSKTS LWKEM QYNRD IiIRF KFANS IIKSG GLGYK WSEiS FKPmN>
              ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^ ^

                        310  320  330  340  350
                         *    *    *    *
C486G4p       YQYTY TRDGE EVTAH TTCSV NGIND FNYNG GSLPT DFVIS KYEVI KENSF

SA11G4p          305  310  315  320  325  330  335  340  345  350
[ 3506 ]      YQYTY TRDGE EVTAH TTCSV NGVND FNYNG GSLPT DFVIS KYEVI KENSF>
              ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RRVVP4p          305  310  315  320  325  330  335  340  345  350
[ 3198 ]      YQYTY TRDGE dVTAH TTCSV NGmND FNfNG GSLPT DFiIS rYEVI KENSy>
              ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

DS-1p         300  305  310  315  320  325  330  335  340  345
[ 2870 ]      YQYsY sRDGE qVTAH TTCSV NGvNn FsYNG GSLPT DFsIS rYEVI KENSy>
              ^^^ ^ ^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^^^ ^^^^^ ^^ ^^ ^^^^^ ^^^^^

M37p          300  305  310  315  320  325  330  335  340  345
[ 2844 ]      YQYnY lRDGE qVTAH TTCSV NGvNn FsYNG GSLPT DFsvS rYEVI KENSy>
              ^^^ ^ ^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^^^ ^^^^^ ^^ ^^ ^^^^^ ^^^^^

KUp           300  305  310  315  320  325  330  335  340  345
[ 2837 ]      YQYnY lRDGE qVTAH TTCSV NGvNn FsYNG GSLPT DFsvS rYEVI KENSy>
              ^^^ ^ ^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^^^ ^^^^^ ^^ ^^ ^^^^^ ^^^^^

ST3p          300  305  310  315  320  325  330  335  340  345
[ 2836 ]      YQYnY lRDGE qVTAH TTCSV NGvNn FsYNG GlLPT hFsvS rYEVI KENSy>
              ^^^ ^ ^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^^^ ^ ^^ ^^^^^ ^^^^^
```

Figure 3 (Sheet 7 of 17)

```
K8p                305   310   315   320   325   330   335   340   345   350
[ 2735 ]           YQYTY TRDeE EVTAH TTCSV NGvND FNYNG GtLPT DFaIS rfEVI KENSy>
                   ^^^^^ ^^^ ^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^^^ ^^ ^^ ^^^^^ ^^^^^

                         360   370   380   390   400
                          *     *     *     *     *
C486G4p            VYIDY WDDSQ AFRNM VYVRS LAADL NSVMC TGGDY SFAIP VGNYP VMTGG

                   355   360   365   370   375   380   385   390   395   400
SA11G4p            VYIDY WDDSQ AFRNM VYVRS LAADL NSVMC TGGDY SFAlP VGNYP VMTGG>
[ 3506 ]           ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

                   355   360   365   370   375   380   385   390   395   400
RRVVP4p            VYvDY WDDSQ AFRNM VYVRS LAAnL NSViC TGGDY SFAlP VGqwP VMTGG>
[ 3198 ]           ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^ ^ ^^^^^

                   350   355   360   365   370   375   380   385   390   395
DS-1p              VYIDY WDDSk AFRNM VYVRS LAAnL NSVkC TGGsY nFrlP VGkwP iMnGG>
[ 2870 ]           ^^^^^ ^^^^  ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^^^ ^ ^^^ ^^ ^ ^^ ^^

                   350   355   360   365   370   375   380   385   390   395
M37p               VYvDY WDDSQ AFRNM VYVRS LAAnL NSVkC TGGnY nFqlP VGawP VMsGG>
[ 2844 ]           ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^^^^^ ^^ ^^ ^ ^^ ^^

                   350   355   360   365   370   375   380   385   390   395
KUp                VYvDY WDDSk AFRNM VYVRS LAAnL NSVkC TGGsY dFsIP VGawP VMnGG>
[ 2837 ]           ^^^^^ ^^^^  ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^^^ ^ ^^^ ^^ ^ ^^ ^^

                   350   355   360   365   370   375   380   385   390   395
ST3p               VYvnY WDDSQ AlRNM VYVRS LAAnL NSVkC TGGnY nFqlP VGawP VMsGG>
[ 2836 ]           ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^^^^^ ^^ ^^ ^ ^^ ^^
```

Figure 3 (Sheet 8 of 17)

```
K8p                355  360  365  370  375  380  385  390  395  400
[ 2735 ]       VYVDY WDDSQ AFRNM VYVRS LAAnL NdVvC TGGsY SFAlP VGNhP VMsGG>
               ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^^^ ^^^ ^ ^^^^^ ^^^ ^ ^^ ^^

                         410       420       430       440       450
                          *         *         *         *         *
C486G4p        AVSLH SAGVT LSTQF TDFVS LNSLR FRFRL SVEEP PFSIL RTRVS GLYGL

SA11G4p            405  410  415  420  425  430  435  440  445  450
[ 3506 ]       AVSLH SAGVT LSTQF TDFVS LNSLR FRFRL SVEEP PFSIL RTRVS GLYGL>
               ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RRVVP4p            405  410  415  420  425  430  435  440  445  450
[ 3198 ]       AVSLH SAGVT LSTQF TDFVS fNSLR FRFRL tVEEP sFSIt RTRVg GLYGL>
               ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^ ^^^ ^^^^ ^^^^^

DS-1p          400  405  410  415  420  425  430  435  440  445
[ 2870 ]       AVSLH fAGVT LSTQF TDFVS LNSLR FRFsL tVdEP sFSIL RTRti nLYGL>
               ^^^^^ ^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^ ^ ^^^^ ^^^ ^^^ ^^^^

M37p           400  405  410  415  420  425  430  435  440  445
[ 2844 ]       AVSLH fAGVT LSTeF TDFVS LNSLR FRFsL tVEEP PFSIL RTRVS GLYGL>
               ^^^^^ ^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^ ^ ^^^^ ^^^^^ ^^^^^ ^^^^^

KUp            400  405  410  415  420  425  430  435  440  445
[ 2837 ]       AVSLH fAGVT LSTQF TDFVS LNSLR FRFsL tVdEP sFSIL RTRtv nLYGL>
               ^^^^^ ^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^ ^ ^^^^ ^^^^ ^^^ ^^^^

ST3p           400  405  410  415  420  425  430  435  440  445
[ 2836 ]       AVSLH fAGVT LSTkF TDFVS LNSLR FRFsL tVEEP PFSIL RTRVS GLYGL>
               ^^^^^ ^^^^ ^^^ ^ ^^^^^ ^^^^^ ^^^^^ ^^^ ^ ^^^^ ^^^^^ ^^^^^ ^^^^^
```

Figure 3 (Sheet 9 of 17)

```
                    405   410   415   420   425   430   435   440   445   450
K8p                 AVtLt SAGVT LSTQy TDyVS LNSLq FRFRL aVsEP sFSIs RTRmS GiVGL>
[ 2735 ]            ^^ ^  ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^^  ^^^  ^^^^^ ^^^^^

                          460   470   480   490   500
                            *     *     *     *
C486G4p             PAAKP NNSQE YYEIA GRFSL ISLVP SNDDY QTPII NSVTV RQDLE RQLGE

                    455   460   465   470   475   480   485   490   495   500
SA11G4p             PAAKP NNSQE YYEIA GRFSL ISLVP lNDDY QTPIm NSVTV RQDLE RQLGE>
[ 3506 ]            ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

                    455   460   465   470   475   480   485   490   495   500
RRVVP4p             PAAyP NNgkE YYEvA GR1SL ISLVP SNDDY QTPIt NSVTV RQDLE RQLGE>
[ 3198 ]            ^^^ ^ ^^  ^ ^^^^^ ^^^^^ ^^^^^ ^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

                  450   455   460   465   470   475   480   485   490   495
DS-1p               PAAnP NNgnE YYEms GRFSL ISLVq tNDDY QTPIm NSVTV RQDLE RQLnd>
[ 2870 ]            ^^^ ^ ^^  ^ ^^^^  ^^^^^ ^^^^  ^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^ ^

                  450   455   460   465   470   475   480   485   490   495
M37p                PAfnP NsghE YYEIA GRFSf I1LVP SNDDY QTPIm NSVTV RQDLE RQLGd>
[ 2844 ]            ^^  ^ ^  ^^ ^^^^^ ^^^^^ ^ ^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

                  450   455   460   465   470   475   480   485   490   495
KUp                 PAAnP NNgnE YYEIs GRFSL ISLVP tNDDY QTPIm NSVTV RQDLE RQLtd>
[ 2837 ]            ^^^ ^ ^^  ^ ^^^^  ^^^^^ ^^^^^ ^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^ ^

                  450   455   460   465   470   475   480   485   490   495
ST3p                PAsnP NsghE YYEIA GRFSL ISLVP SNDDY QTPIm NSiTV RQDLE RQLGd>
[ 2836 ]            ^^  ^ ^  ^^ ^^^^^ ^^^^^ ^^^^^ ^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^
```

Figure 3 (Sheet 10 of 17)

```
K8p                455  460  465  470  475  480  485  490  495  500
[ 2735 ]           PAvnP NNSaE YYEIA GRFSL ISLVP tNDDY QTPIa NSVTV RQDLE RQLGE>
                   ^^ ^ ^^^ ^ ^^^^^ ^^^^^ ^^^^^ ^^^^ ^^^^ ^^^^^ ^^^^^ ^^^^^

                        510  520  530  540  550
                          *    *    *    *
C486G4p            LRDEF NNLSQ QIAMS QLIDL ALLPL DMFSM FSGIK STIDA AKSMA TNVMK

SA11G4p            505  510  515  520  525  530  535  540  545  550
[ 3506 ]           LRDEF NNLSQ QIAMS QLIDL ALLPL DMFSM FSGIK STIDA AKSMA TNVMK>
                   ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RRVVP4p            505  510  515  520  525  530  535  540  545  550
[ 3198 ]           LReEF NaLSQ eIAMS QLIyL ALLPL DMFSM FSGIK STIDA AKSMA TsVMK>
                   ^^^^^ ^ ^^^ ^^^^^ ^^^ ^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^^^

DS-1p              500  505  510  515  520  525  530  535  540  545
[ 2870 ]           LReEF NsLSQ eIAMS QLIDL ALLPL DMFSM FSGIK STIDl tKSMA TsVMK>
                   ^^^^^ ^ ^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^ ^^^^   ^^^^ ^ ^^^

M37p               500  505  510  515  520  525  530  535  540  545
[ 2844 ]           LReEF NsLSQ eIAMt QLIDL ALLPL DMFSM FSGIK STIDA AKSMA TkVMK>
                   ^^^^^ ^ ^^^ ^^^^  ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^^^

KUp                500  505  510  515  520  525  530  535  540  545
[ 2837 ]           LReEF NsLSQ eIAMS QLIDL ALLPL DMFSM FSelK STIDl tKSMA TsVMK>
                   ^^^^^ ^ ^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^ ^^ ^^^^   ^^^^ ^ ^^^

ST3p               500  505  510  515  520  525  530  535  540  545
[ 2836 ]           LReEF NsLSQ eIAit QLIDL ALLPL DMFSM FSGIK STIDA AKSMA TkVMK>
                   ^^^^^ ^ ^^^ ^^^^  ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^^^
```

Figure 3 (Sheet 11 of 17)

```
K8p                505   510   515   520   525   530   535   540   545   550
[ 2735 ]       LReEF NsLSQ eIAvS QLIDL AtLPL DMFSM FSGIK STveA vKSMt TNVMK>
               ^^^^^ ^ ^^^ ^^^^^ ^^^^^ ^ ^^^ ^^^^^ ^^^^^ ^^^^^ ^^^ ^^^^^

                         560   570   580   590   600
                           *     *     *     *
C486G4p        RFKKS SLANS VSTLT DSLSD AASSI SRSAS VRSVS STASA WTEVS NITSD

SA11G4p            555   560   565   570   575   580   585   590   595   600
[ 3506 ]       RFKKS SLANS VSTLT DSLSD AASSI SRSAS VRSVS STASA WTEVS NIaSD>
               ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^ ^^

RRVVP4p            555   560   565   570   575   580   585   590   595   600
[ 3198 ]       kFKKS gLANS VSTLT DSLSD AASSI SRgAS iRSVg SsASA WTdVS tqitD>
               ^^^^^ ^^^^ ^^^^^ ^^^^^ ^^^^^ ^^ ^^ ^^^^ ^ ^^^ ^^^^^ ^

DS-1p          550   555   560   565   570   575   580   585   590   595
[ 2870 ]       kFrKS kLAtS iSemT nSLSD AASSa SRSAS iRSni STiSn WTntS ksvSn>
               ^^^^^ ^^ ^ ^^ ^^ ^^^^^ ^^^^ ^^^^^ ^^^ ^^ ^ ^^ ^ ^^

M37p           550   555   560   565   570   575   580   585   590   595
[ 2844 ]       kFKrS gLAtS iSeLT gSLSn AASSI SRSsS iRSni SsiSv WTdVS eqiag>
               ^^^^^ ^^ ^ ^^ ^^ ^^^^ ^^^^^ ^^^ ^ ^^^ ^ ^ ^^^^^

KUp            550   555   560   565   570   575   580   585   590   595
[ 2837 ]       kFrKS kLAtS iSemT hSLSD AASSa SRSvS iRSni STiSn WTnVS NdvSn>
               ^^^^^ ^^ ^ ^^ ^^ ^^^^ ^^^^ ^^^ ^ ^^^ ^^ ^ ^^ ^^ ^ ^^

ST3p           550   555   560   565   570   575   580   585   590   595
[ 2836 ]       kFKrS gLAtS iSeLT rSLSn AASSI SRSsS iRSni SsvSe WTdVS eqiag>
               ^^^^^ ^^ ^ ^^ ^^ ^^^^ ^^^^^ ^^^ ^ ^^^ ^ ^ ^^^^^
```

Figure 3 (Sheet 12 of 17)

```
K8p              555   560   565   570   575     580   585   590   595
[ 2735 ]     RFKtS SLANa iSdLT snmSe AASSv -RltS VRSVg titlp rarVS lqvgD>
             ^^^ ^ ^^^^  ^^ ^^   ^^^ ^^^^^  ^  ^ ^^^^        ^  ^^     ^

                       610         620         630         640         650
                         *           *           *           *           *
C486G4p      INVTT SSIST QTSTI SRRLR LKEMA TQTDG MNFDD ISAAV LKTKI DKSTQ

SA11G4p        605   610   615   620   625   630   635   640   645   650
[ 3506 ]     INVTT SSIST QTSTI SRRLR LKEMA TQTDG MNFDD ISAAV LKTKI DKSTQ>
             ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RRVVP4p        605   610   615   620   625   630   635   640   645   650
[ 3198 ]     vsssv SSIST QTSTI SRRLR LKEMA TQTeG MNFDD ISAAV LKTKI DrSTQ>
             ^     ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

DS-1p      600   605   610   615   620   625   630   635   640   645
[ 2870 ]     vtdsv ndIST QTSTI SkkLR LrEMi TQTeG lsFDD ISAAV LKTKI DmSTQ>
             ^       ^^^ ^^^^^ ^^^^^ ^^^^  ^^^^^ ^ ^^^ ^^^^^ ^^^^^ ^ ^^^

M37p       600   605   610   615   620   625   630   635   640   645
[ 2844 ]     ssdsv SnIST QmSaI SRRLR LrEit TQTeG MNFDD ISAAV LKTKI DrSTh>
                   ^ ^^^ ^ ^ ^ ^^^^^ ^^^^  ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

KUp        600   605   610   615   620   625   630   635   640   645
[ 2837 ]     vtnsl SdIST QTSTI SknLR LKEMi TQTeG MsFDD ISAAV LKTKI DmSTQ>
             ^     ^ ^^^ ^^^^^ ^^ ^^ ^^^^  ^^^^^ ^ ^^^ ^^^^^ ^^^^^ ^ ^^^

ST3p       600   605   610   615   620   625   630   635   640   645
[ 2836 ]     ssdsv rnIST QiSaI SRRLR LrEit TQTeG MNFiD ISAAV LKTKI DrSTh>
                   ^^^ ^ ^ ^ ^^^^^ ^^^^  ^^^^^ ^^^ ^ ^^^^^ ^^^^^ ^^^^^
```

Figure 3 (Sheet 13 of 17)

```
K8p          600   605   610   615   620   625   630   635   640   645
[ 2735 ]   dlrsm qdvST QvSnv SRnLR LKEft TQTDt lsFDD ISAAV LKTKl DKSTQ>
                ^^^ ^ ^ ^ ^^ ^^ ^^^  ^^^^  ^ ^^^ ^^^^^ ^^^^^ ^^^^^

                      660       670       680       690       700
                        *         *         *         *         *
C486G4p      LNTNT LPEIV TEASE KFIPN RAYRV IKDDE VLEAS TDGKY FAYKV ETILK

                                                                  i
                                                                  |
SA11G4p        655   660   665   670   675   680   685   690   695   700
[ 3506 ]   LNTNT LPEIV TEASE KFIPN RAYRV IKDDE VLEAS iDGKY FAYKV ETfee>
           ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^  ^^^^ ^^^^^ ^^

                                                                  i
                                                                  |
RRVVP4p        655   660   665   670   675   680   685   690   695   700
[ 3198 ]   ispNT LPdIV TEASE KFIPN RAYRV InnDE VfEAg TDGrY FAYrV ETfde>
           ^ ^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^^^ ^^^^  ^^^^^ ^^^^^ ^^

                                                                  i
                                                                  |
DS-1p          650   655   660   665   670   675   680   685   690   695
[ 2870 ]   igkNT LPdIV TEASE KFIPk RsYRV lKDDE VmEin TeGKf FAYKV dTlne>
           ^ ^^ ^^^^^ ^^^^^ ^^^^^ ^^^^  ^ ^^^ ^^^^^ ^^^   ^^^^^ ^^^^^ ^^^

                                                                  v
                                                                  |
M37p           650   655   660   665   670   675   680   685   690   695
[ 2844 ]   ispdT LPdIm TEsSk KFIPk RAYRV lKDDE VmEAd vDGKf FAYKV dTfee>
           ^ ^^ ^^^^^ ^^ ^  ^^^^  ^^^^^ ^^^^^ ^^^^  ^^^^ ^^^^^ ^^^^^ ^^
```

Figure 3 (Sheet 14 of 17)

```
                                                                          v
KUp          650   655   660   665   670   675   680   685   690   695    |
[ 2837 ]       igkNT LPdIV TEASE KFIPk RsYRi lKDDE VmEin TeGKv FAYKi dTlne>
               ^  ^^ ^^^^^ ^^^^^ ^^^^  ^ ^^^ ^^^^^ ^^^   ^^^^  ^^^^^ ^^^

                                                                          v
ST3p         650   655   660   665   670   675   680   685   690   695    |
[ 2836 ]       irpdT LPdIi TEsSE KFIPk RAYRV lKDDE VmEAd vDGKf FAYKV dTfee>
               ^  ^^ ^^^^^ ^^ ^^ ^^^^  ^^^^^ ^^^^^ ^^^^   ^^^^ ^^^^^ ^^

                                                                          i
K8p          650   655   660   665   670   675   680   685   690   695    |
[ 2735 ]       isqqT mPdIi aEsSE KFIPk RsYRi vdeDi rfEtg iDGtf yAYKV dTfne>
               ^   ^ ^^^^^   ^ ^^ ^^^^ ^ ^^^ ^ ^^   ^^    ^^ ^ ^^^^^ ^^

                         710         720         730         740         750
                          *           *           *           *           *
C486G4p        RFHSM YKFAD LVTDS PVISA IIDFK TLKNL NDNYG ISRQQ ALNLL RSDPR

SA11G4p          705   710   715   720   725   730   735   740   745   750
[ 3506 ]       pFd-v qKFAD LVTDS PVISA IIDFK TLKNL NDNYG ISRQQ ALNLL RSDPR>
                ^  ^  ^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

RRVVP4p          705   710   715   720   725   730   735   740   745   750
[ 3198 ]       pFd-v qKFAD LVTDS PVISA IIDFK TLKNL NDNYG ISRQQ AfNLL RSDPR>
                ^  ^  ^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^

DS-1p                  705   710   715   720   725   730   735   740   745
```

Figure 3 (Sheet 15 of 17)

SUBSTITUTE SHEET

```
[ 2870 ]    pFd-i nKFAe LVTDS PVISA IIDFK TLKNL NDNYG ItRie AfNLi kSnPn>
             ^    ^  ^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^  ^  ^ ^^^^^ ^^^^

M37p              705   710   715   720   725   730   735   740   745
[ 2844 ]    pFd-v dKFvD LVTDS PVISA IIDFK TLKNL NDNYG ItRsQ ALdLi RSDPR>
             ^    ^  ^^ ^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^  ^  ^ ^^^^^ ^^^^^

KUp               705   710   715   720   725   730   735   740   745
[ 2837 ]    pFd-v nKFAe LVTnS PVISA IIDFK TLKNL NDNYG ItRie ALNLi kSnPn>
             ^    ^  ^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^  ^  ^ ^^^^^ ^^^^

ST3p              705   710   715   720   725   730   735   740   745
[ 2836 ]    pFd-v dKFvD LVTDS PVISA IIDFK TLKNL NDNYG ItRsQ ALdLi RSDPR>
             ^    ^  ^^ ^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^  ^  ^ ^^^^^ ^^^^^

K8p               705   710   715   720   725   730   735   740   745
[ 2735 ]    pFd-M erFnk LiTDS PVlSA IIDFK TLKNL NDNYG ItkkQ AmeLL hSnPk>
             ^    ^  ^^   ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^  ^  ^ ^^ ^^ ^^^^^


                       760         770
                        *           *
C486G4p     VLREF INQDN PIIRN RIESL IMQCR L

SA11G4p       755   760   765   770   775
[ 3506 ]    VLREF INQDN PIIRN RIESL IMQCR L>
            ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^

RRVVP4p       755   760   765   770   775
[ 3198 ]    VLREF INQDN PIIRN RIEqL IMQCR L>
            ^^^^^ ^^^^^ ^^^^^ ^^^ ^ ^^^^^ ^
```

Figure 3 (Sheet 16 of 17)

```
DS-1p      750   755   760   765   770   775
[ 2870 ]     VLRnF INQnN PIIRN RIEqL I1QCk L>
             ^^^ ^ ^^^^^ ^^^^^ ^^^ ^ ^^^^^ ^

M37p       750   755   760   765   770   775
[ 2844 ]     VLRdF INQnN PIIkN RIEqL I1QCR L>
             ^^^^^ ^^^^^ ^^^^^ ^^^ ^ ^^^^^ ^

KUp        750   755   760   765   770   775
[ 2837 ]     VLRnF INQnN PIIRN RIEqL I1QCk L>
             ^^^ ^ ^^^^^ ^^^^^ ^^^ ^ ^^^^^ ^

ST3p       750   755   760   765   770   775
[ 2836 ]     VLRdF INQnN PIIkN RIEqL I1QCR L>
             ^^^^^ ^^^^^ ^^^^^ ^^^ ^ ^^^^^ ^

K8p        750   755   760   765   770   775
[ 2735 ]     tLkEF INnnN PIIRN RIEnL IsQCR L>
             ^^^^ ^^ ^^ ^^^^^ ^^^ ^ ^ ^^^ ^
```

Figure 3 (Sheet 17 of 17)

Sequence Range: 1 to 2363

```
            10          20          30          40
             *           *           *           *
GGC TAT AAA ATG GCT TCA CTC ATT TAT AGA CAG TTG CTT ACT AAT
            Met Ala Ser Leu Ile Tyr Arg Gln Leu Leu Thr Asn>

    50          60          70          80          90
     *           *           *           *           *
TCA TAC ACA GTA GAA CTT TCA GAT GAA ATC CAA GAA ATT GGA TCG
Ser Tyr Thr Val Glu Leu Ser Asp Glu Ile Gln Glu Ile Gly Ser>

           100         110         120         130
            *           *           *           *
ACT AAG ACT CAA AAC GTT ACC GTT AAT CCA GGA CCG TTC GCG CAA
Thr Lys Thr Gln Asn Val Thr Val Asn Pro Gly Pro Phe Ala Gln>

   140         150         160         170         180
    *           *           *           *           *
ACA AAT TAC GCT TCA GTT AAT TGG GGA CCT GGT GAA ACG AAT GAC
Thr Asn Tyr Ala Ser Val Asn Trp Gly Pro Gly Glu Thr Asn Asp>

           190         200         210         220
            *           *           *           *
TCA ACT ACA GTT GAA CCA GTG CTT GAT GGA CCA TAT CAA CCA ACG
Ser Thr Thr Val Glu Pro Val Leu Asp Gly Pro Tyr Gln Pro Thr>

   230         240         250         260         270
    *           *           *           *           *
ACT TTT AAT CCA CCT GTA AGT TAT TGG ATG TTG TTA GCA CCA ACG
Thr Phe Asn Pro Pro Val Ser Tyr Trp Met Leu Leu Ala Pro Thr>
```

Figure 4 (Sheet 1 of 9)

```
                280                 290                 300                 310
                 *                   *                   *                   *
AAC GCG GGG GTG GTA GAT CAA GGT ACG AAC AAT ACA AAC AGA TGG
Asn Ala Gly Val Val Asp Gln Gly Thr Asn Asn Thr Asn Arg Trp>

    320                 330                 340                 350                 360
     *                   *                   *                   *                   *
TTA GCG ACA ATA TTA ATT AAA CCA AAT GTA CAG CAA GTT GAG CGA
Leu Ala Thr Ile Leu Ile Lys Pro Asn Val Gln Gln Val Glu Arg>

        370                 380                 390                 400
         *                   *                   *                   *
ACA TAT ACA TTA TTT GGG CAA CAA GTT CAA GTA ACA GTA TCA AAT
Thr Tyr Thr Leu Phe Gly Gln Gln Val Gln Val Thr Val Ser Asn>

    410                 420                 430                 440                 450
     *                   *                   *                   *                   *
GAT TCA CAG ACA AAG TGG AAG TTT GTG GAT CTA AGT AAG CAG ACA
Asp Ser Gln Thr Lys Trp Lys Phe Val Asp Leu Ser Lys Gln Thr>

        460                 470                 480                 490
         *                   *                   *                   *
CAA GAT GGT AAT TAT TCA CAA CAC GGT CCT CTA CTG TCA ACA CCG
Gln Asp Gly Asn Tyr Ser Gln His Gly Pro Leu Leu Ser Thr Pro>

    500                 510                 520                 530                 540
     *                   *                   *                   *                   *
AAA CTG TAT GGA GTG ATG AAA CAT GGA GGT AAA ATT TAC ACT TAT
Lys Leu Tyr Gly Val Met Lys His Gly Gly Lys Ile Tyr Thr Tyr>
```

Figure 4 (Sheet 2 of 9)

```
                550         560         570         580
                 *           *           *           *
AAT GGA GAG ACA CCG AAC GCA ACT ACT GGT TAC TAC TCT ACA ACT
Asn Gly Glu Thr Pro Asn Ala Thr Thr Gly Tyr Tyr Ser Thr Thr>

    590         600         610         620         630
     *           *           *           *           *
AAC TTT GAC ACT GTA AAC ATG ACA GCA TAT TGT GAT TTT TAT ATA
Asn Phe Asp Thr Val Asn Met Thr Ala Tyr Cys Asp Phe Tyr Ile>

        640         650         660         670
         *           *           *           *
ATT CCA TTA GCA CAA GAA GCA AAA TGC ACT GAA TAC ATA AAT AAT
Ile Pro Leu Ala Gln Glu Ala Lys Cys Thr Glu Tyr Ile Asn Asn>

    680         690         700         710         720
     *           *           *           *           *
GGA TTA CCA CCA ATA CAA AAT ACG AGA AAT ATC GTA CCA GTT TCG
Gly Leu Pro Pro Ile Gln Asn Thr Arg Asn Ile Val Pro Val Ser>

        730         740         750         760
         *           *           *           *
ATA GTA TCA AGG AAT ATT GTA TAT ACA AGA GCA CAA CCT AAT CAA
Ile Val Ser Arg Asn Ile Val Tyr Thr Arg Ala Gln Pro Asn Gln>

    770         780         790         800         810
     *           *           *           *           *
GAC ATA GTG GTA TCA AAA ACT TCA TTA TGG AAA GAG ATG CAA TAT
Asp Ile Val Val Ser Lys Thr Ser Leu Trp Lys Glu Met Gln Tyr>
```

Figure 4 (Sheet 3 of 9)

```
                820                 830                 840                 850
                 *                   *                   *                   *
AAT AGA GAT ATA GTG ATA AGA TTT AAA TTT GCT AAC TCA ATC ATA
Asn Arg Asp Ile Val Ile Arg Phe Lys Phe Ala Asn Ser Ile Ile>

    860         870         880         890         900
     *           *           *           *           *
AAA TCA GGG GGA TTG GGA TAT AAA TGG TCA GAA GTG TCA TTT AAA
Lys Ser Gly Gly Leu Gly Tyr Lys Trp Ser Glu Val Ser Phe Lys>

        910         920         930         940
         *           *           *           *
CCA GCT AAT TAT CAG TAC ACA TAT ACC AGA GAT GGT GAA GAA GTT
Pro Ala Asn Tyr Gln Tyr Thr Tyr Thr Arg Asp Gly Glu Glu Val>

    950         960         970         980         990
     *           *           *           *           *
ACT GCA CAT ACT ACG TGT TCA GTA AAT GGA ATA AAT GAT TTT AAT
Thr Ala His Thr Thr Cys Ser Val Asn Gly Ile Asn Asp Phe Asn>

        1000        1010        1020        1030
         *           *           *           *
TAT AAT GGT GGA TCA TTA CCG ACT GAT TTC GTA ATA TCA AAA TAT
Tyr Asn Gly Gly Ser Leu Pro Thr Asp Phe Val Ile Ser Lys Tyr>

    1040        1050        1060        1070        1080
     *           *           *           *           *
GAA GTG ATT AAG GAA AAT TCT TTT GTG TAT ATA GAC TAC TGG GAC
Glu Val Ile Lys Glu Asn Ser Phe Val Tyr Ile Asp Tyr Trp Asp>
```

Figure 4 (Sheet 4 of 9)

```
      1090        1100        1110        1120
        *           *           *           *
GAT TCA CAA GCA TTT AGA AAC ATG GTA TAT GTA CGC TCG TTG GCA
Asp Ser Gln Ala Phe Arg Asn Met Val Tyr Val Arg Ser Leu Ala>

  1130        1140        1150        1160        1170
    *           *           *           *           *
GCC GAT TTA AAT TCG GTA ATG TGT ACA GGA GGT GAC TAT AGT TTT
Ala Asp Leu Asn Ser Val Met Cys Thr Gly Gly Asp Tyr Ser Phe>

      1180        1190        1200        1210
        *           *           *           *
GCG ATT CCA GTT GGT AAT TAT CCA GTT ATG ACT GGG GGT GCT GTG
Ala Ile Pro Val Gly Asn Tyr Pro Val Met Thr Gly Gly Ala Val>

  1220        1230        1240        1250        1260
    *           *           *           *           *
TCA TTG CAT TCA GCA GGT GTA ACT TTA TCA ACG CAG TTT ACA GAT
Ser Leu His Ser Ala Gly Val Thr Leu Ser Thr Gln Phe Thr Asp>

      1270        1280        1290        1300
        *           *           *           *
TTC GTA TCA TTA AAT TCA CTG AGA TTT AGA TTT AGA TTA TCA GTA
Phe Val Ser Leu Asn Ser Leu Arg Phe Arg Phe Arg Leu Ser Val>

  1310        1320        1330        1340        1350
    *           *           *           *           *
GAA GAA CCG CCG TTC TCA ATT CTA CGG ACC AGA GTT AGT GGA TTG
Glu Glu Pro Pro Phe Ser Ile Leu Arg Thr Arg Val Ser Gly Leu>
```

Figure 4 (Sheet 5 of 9)

```
          1360          1370          1380          1390
             *             *             *             *
TAT GGA CTT CCA GCG GCA AAA CCG AAT AAT TCA CAA GAA TAT TAT
Tyr Gly Leu Pro Ala Ala Lys Pro Asn Asn Ser Gln Glu Tyr Tyr>

  1400          1410          1420          1430          1440
    *             *             *             *             *
GAG ATA GCT GGG AGA TTT TCA TTA ATA TCA CTC GTA CCG TCA AAT
Glu Ile Ala Gly Arg Phe Ser Leu Ile Ser Leu Val Pro Ser Asn>

          1450          1460          1470          1480
             *             *             *             *
GAT GAT TAT CAG ACA CCA ATA ATA AAT TCA GTC ACT GTA CGA CAA
Asp Asp Tyr Gln Thr Pro Ile Ile Asn Ser Val Thr Val Arg Gln>

  1490          1500          1510          1520          1530
    *             *             *             *             *
GAT TTA GAA CGA CAA TTA GGA GAA CTA AGA GAT GAA TTT AAC AAT
Asp Leu Glu Arg Gln Leu Gly Glu Leu Arg Asp Glu Phe Asn Asn>

          1540          1550          1560          1570
             *             *             *             *
TTA TCA CAA CAA ATC GCT ATG TCA CAA CTG ATA GAT CTT GCG TTA
Leu Ser Gln Gln Ile Ala Met Ser Gln Leu Ile Asp Leu Ala Leu>

  1580          1590          1600          1610          1620
    *             *             *             *             *
CTA CCG TTA GAC ATG TTC TCA ATG TTT TCA GGG ATT AAG AGT ACA
Leu Pro Leu Asp Met Phe Ser Met Phe Ser Gly Ile Lys Ser Thr>
```

Figure 4 (Sheet 6 of 9)

```
      1630        1640        1650        1660
        *           *           *           *
ATT GAC GCA GCG AAG TCT ATG GCG ACG AAT GTA ATG AAG AGA TTT
Ile Asp Ala Ala Lys Ser Met Ala Thr Asn Val Met Lys Arg Phe>

  1670        1680        1690        1700        1710
    *           *           *           *           *
AAA AAG TCA AGT CTC GCT AAC TCA GTG TCA ACG CTC ACT GAT TCA
Lys Lys Ser Ser Leu Ala Asn Ser Val Ser Thr Leu Thr Asp Ser>

      1720        1730        1740        1750
        *           *           *           *
TTG TCT GAT GCA GCA TCA TCA ATT TCT AGA AGT GCA TCG GTT AGA
Leu Ser Asp Ala Ala Ser Ser Ile Ser Arg Ser Ala Ser Val Arg>

  1760        1770        1780        1790        1800
    *           *           *           *           *
TCA GTT AGT TCA ACT GCA TCA GCT TGG ACG GAA GTA TCT AAC ATT
Ser Val Ser Ser Thr Ala Ser Ala Trp Thr Glu Val Ser Asn Ile>

      1810        1820        1830        1840
        *           *           *           *
ACA TCA GAT ATT AAT GTG ACA ACG AGC TCG ATC TCT ACA CAG ACA
Thr Ser Asp Ile Asn Val Thr Thr Ser Ser Ile Ser Thr Gln Thr>

  1850        1860        1870        1880        1890
    *           *           *           *           *
TCA ACA ATA AGC AGA AGG TTA AGA CTA AAA GAA ATG GCG ACT CAA
Ser Thr Ile Ser Arg Arg Leu Arg Leu Lys Glu Met Ala Thr Gln>
```

Figure 4 (Sheet 7 of 9)

```
          1900          1910          1920          1930
             *             *             *             *
ACG GAC GGT ATG AAT TTT GAT GAT ATA TCA GCA GCA GTA CTC AAG
Thr Asp Gly Met Asn Phe Asp Asp Ile Ser Ala Ala Val Leu Lys>

  1940          1950          1960          1970          1980
    *             *             *             *             *
ACT AAA ATT GAT AAA TCA ACC CAG TTA AAT ACA AAT ACA TTG CCG
Thr Lys Ile Asp Lys Ser Thr Gln Leu Asn Thr Asn Thr Leu Pro>

          1990          2000          2010          2020
             *             *             *             *
GAA ATA GTA ACT GAG GCT TCA GAA AAG TTT ATA CCA AAT AGA GCG
Glu Ile Val Thr Glu Ala Ser Glu Lys Phe Ile Pro Asn Arg Ala>

  2030          2040          2050          2060          2070
    *             *             *             *             *
TAC CGT GTA ATT AAA GAT GAT GAA GTG CTA GAG GCT AGT ACT GAT
Tyr Arg Val Ile Lys Asp Asp Glu Val Leu Glu Ala Ser Thr Asp>

          2080          2090          2100          2110
             *             *             *             *
GGT AAA TAT TTC GCT TAC AAA GTT GAA ACC ATT TTG AAG AGA TTC
Gly Lys Tyr Phe Ala Tyr Lys Val Glu Thr Ile Leu Lys Arg Phe>

  2120          2130          2140          2150          2160
    *             *             *             *             *
CAT TCG ATG TAC AAA TTC GCT GAC TTA GTG ACT GAC TCA CCA GTT
His Ser Met Tyr Lys Phe Ala Asp Leu Val Thr Asp Ser Pro Val>
```

Figure 4 (Sheet 8 of 9)

```
                2170        2180        2190        2200
                  *           *           *           *
ATA TCG GCA ATA ATT GAC TTT AAA ACT CTT AAG AAT CTA AAT GAT
Ile Ser Ala Ile Ile Asp Phe Lys Thr Leu Lys Asn Leu Asn Asp>

   2210        2220        2230        2240        2250
     *           *           *           *           *
AAT TAC GGA ATA AGC AGA CAA CAA GCA CTA AAT CTT CTA AGA TCT
Asn Tyr Gly Ile Ser Arg Gln Gln Ala Leu Asn Leu Leu Arg Ser>

      2260        2270        2280        2290
        *           *           *           *
GAT CCG CGA GTA TTA CGT GAA TTT ATT AAT CAG GAT AAT CCA ATA
Asp Pro Arg Val Leu Arg Glu Phe Ile Asn Gln Asp Asn Pro Ile>

   2300        2310        2320        2330        2340
     *           *           *           *           *
ATA CGA AAT AGA ATA GAA AGT TTG ATA ATG CAA TGT CGC TTG TAA
Ile Arg Asn Arg Ile Glu Ser Leu Ile Met Gln Cys Arg Leu End>

      2350        2360
        *           *
GCA ACT GAA CAA GAG GAT GTG AC
```

Figure 4 (Sheet 9 of 9)

Sequence Range: 1 to 1062

```
            10          20          30          40
             *           *           *           *
GGC TTT AAA AGC GAG AAT TTC CGT TTG GCT AGC GGT TAG CTC CTT
CCG AAA TTT TCG CTC TTA AAG GCA AAC CGA TCG CCA ATC GAG GAA
                                                Leu Leu>

    50          60          70          80          90
     *           *           *           *           *
TTA ATG TAT GGT ATT GAA TAT ACC ACA ATT CTA ATC TTC TTG ACA
AAT TAC ATA CCA TAA CTT ATA TGG TGT TAA GAT TAG AAG AAC TGT
Leu Met Tyr Gly Ile Glu Tyr Thr Thr Ile Leu Ile Phe Leu Thr>

          100         110         120         130
            *           *           *           *
TCG ATT ACA TTA TTG AAT TAT ATC TTA AAA TCA ATA ACG AGA ATA
AGC TAA TGT AAT AAC TTA ATA TAG AAT TTT AGT TAT TGC TCT TAT
Ser Ile Thr Leu Leu Asn Tyr Ile Leu Lys Ser Ile Thr Arg Ile>

   140         150         160         170         180
    *           *           *           *           *
ATG GAC TAT ATA ATT TAC AGA TTT CTG CTT ATA GTA GTG ATC TTG
TAC CTG ATA TAT TAA ATG TCT AAA GAC GAA TAT CAT CAC TAG AAC
Met Asp Tyr Ile Ile Tyr Arg Phe Leu Leu Ile Val Val Ile Leu>

          190         200         210         220
            *           *           *           *
GCC ACC ATA ATA AAT GCG CAA AAC TAT GGA GTA AAT TTG CCA ATT
CGG TGG TAT TAT TTA CGC GTT TTG ATA CCT CAT TTA AAC GGT TAA
Ala Thr Ile Ile Asn Ala Gln Asn Tyr Gly Val Asn Leu Pro Ile>
```

Figure 5 (Sheet 1 of 5)

```
                230                 240                 250                 260                 270
                 *                   *                   *                   *                   *
ACA GGT TCA ATG GAT ACT GCG TAT GCA GAC TCT ACA CAA AGT GAG
TGT CCA AGT TAC CTA TGA CGC ATA CGT CTG AGA TGT GTT TCA CTC
Thr Gly Ser Met Asp Thr Ala Tyr Ala Asp Ser Thr Gln Ser Glu>

                280                 290                 300                 310
                 *                   *                   *                   *
CCA TTT TTG ACA TCA ACC CTT TGT TTG TAT TAT CCT GTT GAG GCA
GGT AAA AAC TGT AGT TGG GAA ACA AAC ATA ATA GGA CAA CTC CGT
Pro Phe Leu Thr Ser Thr Leu Cys Leu Tyr Tyr Pro Val Glu Ala>

    320             330                 340                 350                 360
     *               *                   *                   *                   *
TCA AAC GAA ATA GCT GAT ACC GAA TGG AAA GAT ACC TTA TCA CAA
AGT TTG CTT TAT CGA CTA TGG CTT ACC TTT CTA TGG AAT AGT GTT
Ser Asn Glu Ile Ala Asp Thr Glu Trp Lys Asp Thr Leu Ser Gln>

                370                 380                 390                 400
                 *                   *                   *                   *
TTG TTC TTG ACA AAA GGA TGG CCA ACA GGA TCA GTG TAC CTT AAA
AAC AAG AAC TGT TTT CCT ACC GGT TGT CCT AGT CAC ATG GAA TTT
Leu Phe Leu Thr Lys Gly Trp Pro Thr Gly Ser Val Tyr Leu Lys>

    410             420                 430                 440                 450
     *               *                   *                   *                   *
GAA TAT GCT GAT ATA GCG GCC TTT TCA GTG GAA CCA CAG TTA TAC
CTT ATA CGA CTA TAT CGC CGG AAA AGT CAC CTT GGT GTC AAT ATG
Glu Tyr Ala Asp Ile Ala Ala Phe Ser Val Glu Pro Gln Leu Tyr>
```

Figure 5 (Sheet 2 of 5)

```
                460         470         480         490
                 *           *           *           *
TGC GAT TAT AAT TTA GTT TTA ATG AAA TAT GAC TCT ACA CAA GAA
ACG CTA ATA TTA AAT CAA AAT TAC TTT ATA CTG AGA TGT GTT CTT
Cys Asp Tyr Asn Leu Val Leu Met Lys Tyr Asp Ser Thr Gln Glu>

    500         510         520         530         540
     *           *           *           *           *
CTA GAT ATG TCT GAA TTG GCC GAT CTT ATA TTG AAC GAA TGG CTG
GAT CTA TAC AGA CTT AAC CGG CTA GAA TAT AAC TTG CTT ACC GAC
Leu Asp Met Ser Glu Leu Ala Asp Leu Ile Leu Asn Glu Trp Leu>

        550         560         570         580
         *           *           *           *
TGC AAT CCA ATG GAC ATA ACG CTA TAT TAT TAT CAG CAG ACT GAT
ACG TTA GGT TAC CTG TAT TGC GAT ATA ATA ATA GTC GTC TGA CTA
Cys Asn Pro Met Asp Ile Thr Leu Tyr Tyr Tyr Gln Gln Thr Asp>

    590         600         610         620         630
     *           *           *           *           *
GAA GCA AAT AAA TCG ATA TGG ACG GGC TCT TCT TGC ACG GTT AAA
CTT CGT TTA TTT AGC TAT ACC TGC CCG AGA AGA ACG TGC CAA TTT
Glu Ala Asn Lys Ser Ile Trp Thr Gly Ser Ser Cys Thr Val Lys>

        640         650         660         670
         *           *           *           *
GTG TGT CCA TTA AAT ACA CAA ACA CTT GGT ATT GGA TGT CTA ATA
CAC ACA GGT AAT TTA TGT GTT TGT GAA CCA TAA CCT ACA GAT TAT
Val Cys Pro Leu Asn Thr Gln Thr Leu Gly Ile Gly Cys Leu Ile>
```

Figure 5 (Sheet 3 of 5)

```
                680                690                700                710                720
                 *                  *                  *                  *                  *
ACT AAT CCA GAC ACG TTT GAA ACA GTT GCG ACA ATG GAG AAG TTA
TGA TTA GGT CTG TGC AAA CTT TGT CAA CGC TGT TAC CTC TTC AAT
Thr Asn Pro Asp Thr Phe Glu Thr Val Ala Thr Met Glu Lys Leu>

                730                740                750                760
                 *                  *                  *                  *
GTG ATT ACA GAT GTT GTA GAT GGT GTC AAT CAC AAA TTA AAC GTC
CAC TAA TGT CTA CAA CAT CTA CCA CAG TTA GTG TTT AAT TTG CAG
Val Ile Thr Asp Val Val Asp Gly Val Asn His Lys Leu Asn Val>

    770                780                790                800                810
     *                  *                  *                  *                  *
ACA ACG GCA ACG TGC ACC ATA CGC AAC TGT AAA AAG TTA GGA CCA
TGT TGC CGT TGC ACG TGG TAT GCG TTG ACA TTT TTC AAT CCT GGT
Thr Thr Ala Thr Cys Thr Ile Arg Asn Cys Lys Lys Leu Gly Pro>

                820                830                840                850
                 *                  *                  *                  *
AGG GAG AAC GTA GCA GTC ATA CAG GTA GGC GGC GCG AAC ATT TTA
TCC CTC TTG CAT CGT CAG TAT GTC CAT CCG CCG CGC TTG TAA AAT
Arg Glu Asn Val Ala Val Ile Gln Val Gly Gly Ala Asn Ile Leu>

    860                870                880                890                900
     *                  *                  *                  *                  *
GAC ATC ACA GCT GAT CCA ACA ACT ACA CCA CAG ACA GAG ACA ATG
CTG TAG TGT CGA CTA GGT TGT TGA TGT GGT GTC TGT CTC TGT TAC
Asp Ile Thr Ala Asp Pro Thr Thr Thr Pro Gln Thr Glu Thr Met>
```

Figure 5 (Sheet 4 of 5)

```
                910                 920                 930                 940
                 *                   *                   *                   *
ATG CGA ATA AAT TGG AAA AAA TGG TGG CAA GTC TTT TAC ACG GTA
TAC GCT TAT TTA ACC TTT TTT ACC ACC GTT CAG AAA ATG TGC CAT
Met Arg Ile Asn Trp Lys Lys Trp Trp Gln Val Phe Tyr Thr Val>

    950                 960                 970                 980                 990
     *                   *                   *                   *                   *
GTG GAT TAC GTC AAT CAG ATA ATT CAG ACA ATG TCC AAA AGA TCT
CAC CTA ATG CAG TTA GTC TAT TAA GTC TGT TAC AGG TTT TCT AGA
Val Asp Tyr Val Asn Gln Ile Ile Gln Thr Met Ser Lys Arg Ser>

           1000                1010                1020                1030
            *                   *                   *                   *
ACA TCG CTT AAT TCG TCG GCG TTC TAC TAT AGA GTG TAG GTG CAT
TGT AGC GAA TTA AGC AGC CGC AAG ATG ATA TCT CAC ATC CAC GTA
Thr Ser Leu Asn Ser Ser Ala Phe Tyr Tyr Arg Val

    1040                1050                1060
     *                   *                   *
GCT AGA TTA GAG TTG TAT GAT GTG ACC
CGA TCT AAT CTC AAC ATA CTA CAC TGG
```

Figure 5 (Sheet 5 of 5)

Sequence Range: 1 to 354

```
                        10          20          30          40          50
                         *           *           *           *           *
Translatio  GFKSE NFRLA SG*LL LMYGI EYTTI LIFLT SITLL NYILK SITRI MDYII

ROTVP7        10    25    40    55    70    85   100   115   130   145
[ 1586 ]    kiKrE NFRLA SG*LL LMYGI EYTTv LtFLi StiLL NYILK SlTRI MDfII>
            v ^ ^ ^^^^^ ^^v^^ ^^^^^ ^^^^^ ^ ^^  ^  ^^ ^^^^^ ^^^^^ ^^^^^

RORVP7        10    25    40    55    70    85   100   115   130   145
[ 1574 ]    GFKSE NFRLA SG*LL LMYGI EYTTv LtFLi SliLL NYILK SlTRm MDcII>
            ^^^^^ ^^^^^ ^^v^^ ^^^^^ ^^^^^ ^ ^^  ^^ ^^ ^^^^^ ^^^^^ ^^ ^^

PRVOSUVP7     10    25    40    55    70    85   100   115   130   145
[ 1558 ]    GFKrE NFRLA iG*LL LMYGI EYTTv LtFLi Slvfv NYILK SvTRt MDfII>
            ^^^ ^ ^^^^^  ^v^^ ^^^^^ ^^^^^ ^ ^^  ^^ ^^ ^^^^^ ^^^^  ^^^^^

ROHVP7A       10    25    40    55    70    85   100   115   130   145
[ 1544 ]    GFKrE NFRLA nG*LL LMYGI EYTTI LIFLi SIiLL NYILK SvTRI MDYII>
            ^^^ ^ ^^^^^  ^v^^ ^^^^^ ^^^^^ ^^^^  ^^ ^^ ^^^^^ ^^^^^ ^^^^^

PRVPRVP7G     10    25    40    55    70    85   100   115   130   145
[ 1510 ]    GFKrE NFRLA SG*LL LMYGI EYTTv LlyLi SfvLm sYILK tITkm MDYII>
            ^^^ ^ ^^^^^ ^^v^^ ^^^^^ ^^^^^ ^^^^  ^  ^^  ^^^^  ^^^^ ^^^^^

ROB7                    995   980   965   950   935   920   905
[   53 ]                 <flfek lhpsf yq-LT vl*md yflfs SsgRv vsYIc
                          ^^^ v v      v ^^  ^v^v v^^^  ^  ^^ ^ ^^v

PRVPRVVP7                                  60      75    90
[   36 ]                                    L afvi- lIwRr ia-In>
                                            ^  ^^^  v^v^v ^  ^v
```

Figure 6 (Sheet 1 of 7)

```
                          60          70          80          90          100
                           *           *           *           *           *
Translatio  YRFLL IVVIL ATIIN AQNYG VNLPI TGSMD TAYAD STQSE PFLTS TLCLY

ROTVP7       160   175   190   205   220   235   250   265   280   295
[ 1586 ]    YRFLf IiVIL spflr AQNYG iNLPI aGSMD TAYAn STQeE PFLTS TLCLY>
            ^^^^^ ^^^^^   ^   ^^^^^ ^^^^^  ^^^^ ^^^^^ ^^^ ^ ^^^^^ ^^^^^

RORVP7       160   175   190   205   220   235   250   265   280   295
[ 1574 ]    YRFLf IVVIL spllk AQNYG iNLPI TGSMD TAYAn STQeE tFLTS TLCLY>
            ^^^^^ ^^^^^   ^^  ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^ ^  ^^^^ ^^^^^

PRVOSUVP7    160   175   190   205   220   235   250   265   280   295
[ 1558 ]    YRFLL viVvL AplIk AQNYG iNLPI TGSMD TpYmn STtSE tFLTS TLCLY>
            ^^^^^ ^^^^^ ^ ^^  ^^^^^ ^^^^^ ^^^^^ ^ ^ ^ ^^ ^^  ^^^^ ^^^^^

ROHVP7A      160   175   190   205   220   235   250   265   280   295
[ 1544 ]    YRFLL ItVaL faltr AQNYG lNLPI TGSMD avYtn STQeE vFLTS TLCLY>
            ^^^^^ ^ ^ ^ v ^   ^^^^^ ^^^^^ ^^^^^   ^ ^ ^^^ ^  ^^^^ ^^^^^

PRVPRVP7G    160   175   190   205   220   235   250   265   280   295
[ 1510 ]    YRitf IiVvL svlsN AQNYG iNLPI TGSMD TAYAn STQdn nFLsS TLCLY>
            ^^ v^ ^^^^^   ^ ^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^    ^^ ^ ^^^^^

ROB7        890   875
[    53 ]   <qeyyL vsl
             v ^ ^ ^ ^

PRVPRVVP7   105
[    36 ]   l-FLL IV>
              ^^^ ^^
```

Figure 6 (Sheet 2 of 7)

```
                                110                 120                 130                 140                 150
                                 *                   *                   *                   *                   *
Translatio YPVEA SNEIA DTEWK DTLSQ LFLTK GWPTG SVYLK EYADI AAFSV EPQLY

ROTVP7      310  325  340  355  370  385  400  415  430  445
[ 1586 ]    YPtEA atEIn DnsWK DTLSQ LFLTK GWPTe SVYfK EYtnI AsFSV dPQLY>
            ^^ ^^  ^  ^^ ^^^^^ ^^^^^ ^^^^  ^^^^^ ^^ ^^  ^ ^^^ ^^^^^

RORVP7      310  325  340  355  370  385  400  415  430  445
[ 1574 ]    YPtEA atEIn DnsWK DTLSQ LFLTK GWPTG SVYfK EYtDI AsFSV dPQLY>
            ^^ ^^  ^  ^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^ ^^  ^ ^^^ ^^^^^

PRVOSUVP7   310  325  340  355  370  385  400  415  430  445
[ 1558 ]    YPnEA atEIA DTkWt eTLSQ LFLTK GWPTG SVYfK gYADI AsFSV EPQLY>
            ^^v^^ ^^^ ^^ ^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^ ^ ^^^ ^^^^^

ROHVP7A     310  325  340  355  370  385  400  415  430  445
[ 1544 ]    YPtEA StqIn DgdWK DsLSQ mFLTK GWPTG SVYfK EYsnI vdFSV dPQLY>
            ^^ ^^ ^ ^^  ^ ^^^  ^ ^^^^^ ^^^^^ ^^^^^ ^^ ^^  ^^^ ^^^^^

PRVPRVP7G   310  325  340  355  370  385  400  415  430  445
[ 1510 ]    YPsEA ptqIn DnEWK DTLSQ LFLTK GWPTG SVYfn EYsnv leFSi dPkLh>
            ^^ ^^  ^^  ^ ^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^  ^^ ^^ v ^^^ ^^ ^

                    160                 170                 180                 190                 200
                     *                   *                   *                   *                   *
Translatio CDYNL VLMKY DSTQE LDMSE LADLI LNEWL CNPMD ITLYY YQQTD EANKS
```

Figure 6 (Sheet 3 of 7)

```
ROTVP7          460  475  490  505  520  535  550  565  580  595
[ 1586 ]        CDYNv VLMKY DaT1q LDMSE LADLI LNEWL CNPMD ITLYY YQQTD EANKw>
                ^^^^^ ^^^^^ ^ ^v^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^v

RORVP7          460  475  490  505  520  535  550  565  580  595
[ 1574 ]        CDYNv VLMKY DaT1q LDMSE LADLI LNEWL CNPMD IaLYY YQQTD EANKw>
                ^^^^^ ^^^^^ ^ ^v^ ^^^^^ ^^^^^ ^^^^^ ^ ^^^ ^^^^^ ^^^^v

PRVOSUVP7       460  475  490  505  520  535  550  565  580  595
[ 1558 ]        CDYNi VLMKY Dgn1q LDMSE LAgLI LNEWL CNPMD ImLYY YQQTD EANKw>
                ^^^^^ ^^^^^ ^  v^ ^^^^^ ^^ ^^ ^^^^^ ^^^^^ ^ ^^^ ^^^^^ ^^^^v

ROHVP7A         460  475  490  505  520  535  550  565  580  595
[ 1544 ]        CDYNL VLMKY Dqs1k LDMSE LADLI LNEWL CNPMD vTLYY YQQsg EsNKw>
                ^^^^^ ^^^^^ ^  v  ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^ ^ ^^v

PRVPRVP7G       460  475  490  505  520  535  550  565  580  595
[ 1510 ]        CDYNi VLirf aSgeE LDiSE LADLI LNEWL CNPMD ITLYY YQQTg EANKw>
                ^^^^^ ^^^^^ ^ ^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^ ^^^^v

                     210       220       230       240       250
                     *         *         *         *         *
Translatio      IWTGS SCTVK VCPLN TQTLG IGCLI TNPDT FETVA TMEKL VITDV VDGVN

ROTVP7          610  625  640  655  670  685  700  715  730  745
[ 1586 ]        IsmGS SCTiK VCPLN TQTLG IGCLt TdatT FEeVp TaEKL VITDV VDGVN>
                ^v ^^ ^^^^^ ^^^^^ ^^^^^ ^^^^ ^^  ^  ^^ ^  ^ ^^^ ^^^^^ ^^^^^

RORVP7          610  625  640  655  670  685  700  715  730  745
[ 1574 ]        IsmGS SCTiK VCPLN TQTLG IGCLt TdtaT FEeVA TaEKL VITDV VDGVN>
                ^v ^^ ^^^^^ ^^^^^ ^^^^^ ^^^^ ^^  ^  ^^ ^^ ^ ^^^ ^^^^^ ^^^^^
```

Figure 6 (Sheet 4 of 7)

```
PRVOSUVP7     610   625   640   655   670   685   700   715   730   745
[ 1558 ]      IsmGt SCTiK VCPLN TQTLG IGCst Tdins FETVA naEKL aITDV VDGVN>
              ^v ^  ^^^^^ ^^^^^ ^^^^^ ^^^v  ^^v^  ^^^^^   ^^^  ^^^^ ^^^^^

ROHVP7A       610   625   640   655   670   685   700   715   730   745
[ 1544 ]      IsmGS SCTVK VCPLN TQTLG IGCqt TNvDs FEmiA enEKL aIvDV VDGiN>
              ^v ^^ ^^^^^ ^^^^^ ^^^^^ ^^^v  ^^ ^  ^^ ^^   v^^^  ^ ^^ ^^^^^

PRVPRVP7G     610   625   640   655   670   685   700   715   730   745
[ 1510 ]      IsmGS SCTVK VCPLN TQTLG IGCqt TNtaT FETVA dsEKL aIvDV VDsVN>
              ^v ^^ ^^^^^ ^^^^^ ^^^^^ ^^^v  ^^  ^ ^^^^^  v^^^  ^ ^^ ^^ ^^

                      260           270           280           290           300
                       *             *             *             *             *
Translatio    HKLNV TTATC TIRNC KKLGP RENVA VIQVG GANIL DITAD PTTTP QTETM

ROTVP7        760   775   790   805   820   835   850   865   880   895
[ 1586 ]      HKLdV TTATC TIRNC KKLGP RENVA VIQVG GsdIL DITAD PTTaP QTErM>
              ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^^^ ^^^^^ ^^^ ^ ^^^ ^

RORVP7        760   775   790   805   820   835   850   865   880   895
[ 1574 ]      HKLdV TTATC TIRNC KKLGP RENVA VIQVG GsdvL DITAD PTTaP QTErM>
              ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^^^ ^^^^^ ^^^ ^ ^^^ ^

PRVOSUVP7     760   775   790   805   820   835   850   865   880   895
[ 1558 ]      HKLdV TTsTC TIRNC KKLGP RENVA VIQVG GpNIL DITAD PTTaP QTErM>
              ^^^^^ ^^ ^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^^^ ^^^^^ ^^^ ^ ^^^ ^

ROHVP7A       760   775   790   805   820   835   850   865   880   895
[ 1544 ]      HKiNl TTtTC TIRNC KKLGP RENVA VIQVG GsNvL DITAD PTTnP QTErM>
              ^^^^^ ^^ ^^ ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^ ^^^ ^^^^^ ^^^ ^ ^^^ ^
```

Figure 6 (Sheet 5 of 7)

```
PRVPRVP7G    760   775   790   805   820   835   850   865   880   895
[ 1510 ]     HKLdV TstTC TIRNC nKLGP RENVA iIQVG GsNIL DITAn PTTsP QTErM>
             ^^^^^ ^  ^^ ^^^^^  ^^^^ ^^^^^ ^^^^^ ^ ^^^ ^^^^^ ^^^ ^ ^^^ ^

                      310           320           330           340           350
                       *             *             *             *             *
Translatio   MRINW KKWWQ VFYTV VDYVN QIIQT MSKRS TSLNS SAFYY RV*VH ARLEL

ROTVP7       910   925   940   955   970   985  1000  1015  1030  1045
[ 1586 ]     MRINW KKWWQ VFYTV VDYVd QIIQv MSKRS rSLNS aAFYY RV*V* lRLEL>
             ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^  ^^^^^  ^^^^  ^^^^ ^^v^v v^^^^

RORVP7       910   925   940   955   970   985  1000  1015  1030  1045
[ 1574 ]     MRINW KKWWQ VFYTV VDYVN QIIQa MSKRS rSLNS aAFYn Ri*V* lwiEm>
             ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^  ^^^^^  ^^^^  ^^^v ^^v^v v^^^^

PRVOSUVP7    910   925   940   955   970   985  1000  1015  1030  1045
[ 1558 ]     MRINW KrWWQ VFYTi VDYVN QIvQv MSKRS rSLdS aAFYY RV*iy lkLEL>
             ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^  ^^^^^  ^^^^  ^^^^ ^^v^  v^^^^

ROHVP7A      910   925   940   955   970   985  1000  1015   1030  1045
[ 1544 ]     MRvNW KKWWQ VFYTi VDYiN QIvQv MSKRS rSLNS aAFYY RV-*y lRLEL>
             ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^  ^^^^^  ^^^^  ^^^^ ^^ v  v^^^^

PRVPRVP7G    910   925   940   955   970   985  1000  1015  1030  1045
[ 1510 ]     MRvNW KKWWQ VFYTV VDYiN QIvQv MSKRS rSLdS SsFYY RV*iy pkLEL>
             ^^^^^ ^^^^^ ^^^^^ ^^^^^ ^^^^  ^^^^^  ^^^^ ^ ^^^ ^^v^   ^^^^
```

Figure 6 (Sheet 6 of 7)

```
Translatio  YDVT

ROTVP7
[ 1586 ]    YDV>
            ^^^

RORVP7      1060
[ 1574 ]    YDVT>
            ^^^^

PRVOSUVP7   1060
[ 1558 ]    YDVT>
            ^^^^

ROHVP7A
[ 1544 ]    fDVT>
            ^^^^

PRVPRVP7G   1060
[ 1510 ]    YDVT>
            ^^^^
```

Figure 6 (Sheet 7 of 7)

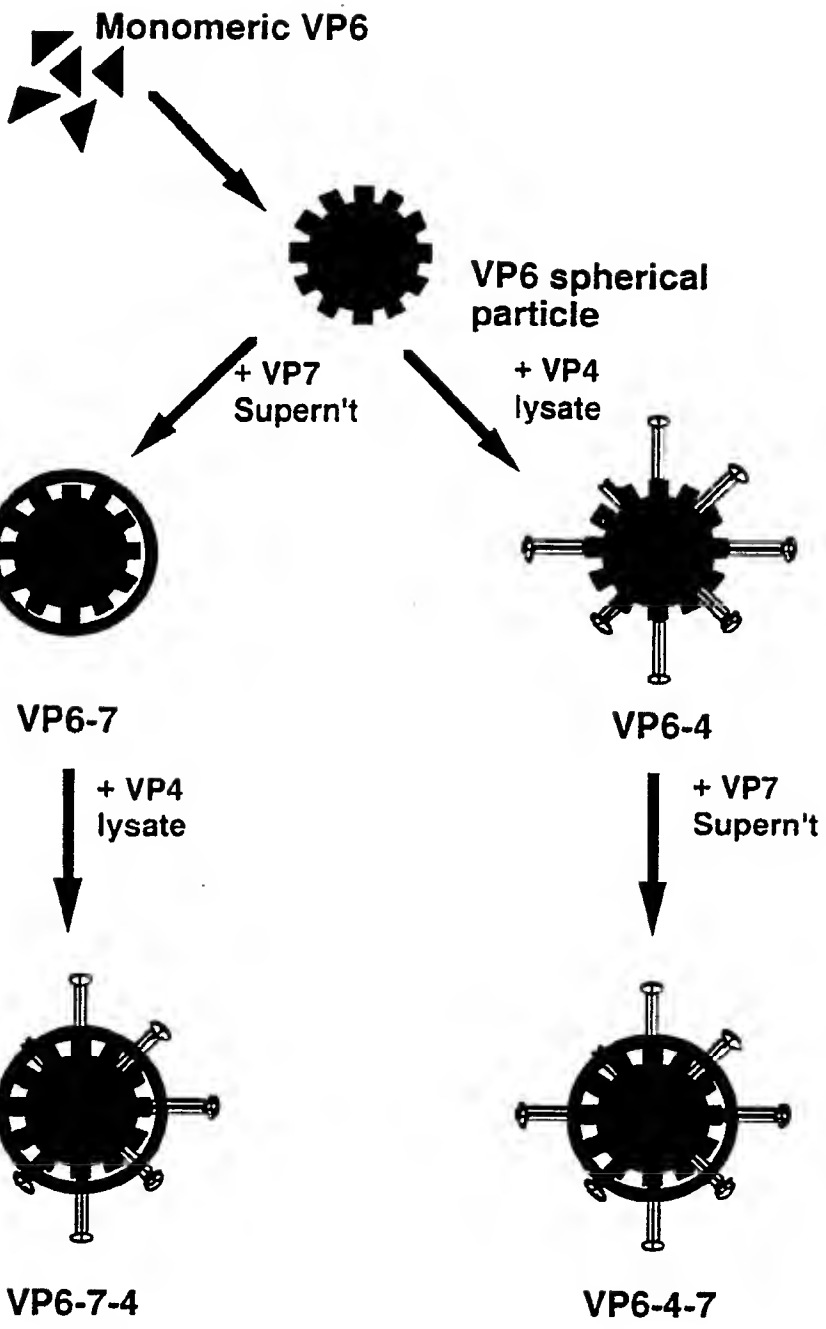
Monomeric VP6
VP6 spherical particle
+ VP7 Supern't
+ VP4 lysate
VP6-7
VP6-4
+ VP4 lysate
+ VP7 Supern't
VP6-7-4
VP6-4-7

FIGURE 7

INTERNATIONAL SEARCH REPO[illegible]

International Application No PCT/CA 91/00376

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 C 12 N 15/46 C 12 N 7/04 A 61 K 39/15

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	C 12 N A 61 K C 07 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	Virology, volume 167, no. 1, November 1988, Academic Press, Inc., K.F.M. Ready et al.: "In vitro assembly of the outer capsid of bovine rotavirus is calcium-dependent", pages 269-273, see the whole article (cited in the application) ---	1,3,6, 10
Y	---	8
O,X	Molecular Immunology, volume 28, no. 3, March 1991, Proceedings of the Vaccine Symposium Celebrating the 75th Anniversary of Connaught Laboratories at the University of Toronto, Pergamon Press (GB), M.J. Redmond et al.: "Rotavirus particles function as immunological carriers for the delivery of peptides from infectious agents and endogenous proteins", pages 269-278, see the whole article --- -/-	1,2,5, 10

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later thao tbe priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of tbe International Search	Date of Mailing of this International Search Report
16-01-1992	18. 02. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	[illegible] Weinberg

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
O,Y	---	8
X	EP,A,0259149 (THE UNIVERSITY OF SASKATCHEWAN) 9 March 1988, see claims	1,2,5, 10
Y	---	8
X	Journal of Virology, volume 64, no. 8, August 1990, American Society for Microbiology (US) H. Brüssow et al.: "Polypeptide composition of rotaviirus empty capsids and their possible use as a subunit vaccine", pages 3635-3642, see the whole article, especially page 3637, lines 5-12	1-7,9, 10,11
Y	---	8
P,X	--- Journal of Virology, volume 65, no. 6, June 1991, American Society for Microbiology, M.K. Ijaz et al.: "Heterotypic passive protection induced by synthetic peptides corresponding to VP7 and VP4 of bovine rotavirus", pages 3106-3113, see the whole article	1-11,14
Y	--- EP,A,0273366 (BAYLOR COLLEGE OF MEDICINE) 6 July 1988, see the whole document	8
L	--- Biosis, BR 41: 19066, see the whole reference (The "L" document is cited to prove that the Symposium mentioned in Mol. Imm. 28, 1991, p 269-278 was held in October 1989	

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claim numbers ** because they relate to subject matter not required to be searched by this Authority, namely:

** Remark: Although claims 18-21 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition

2. [ ] Claim numbers because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. [ ] Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this International application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. CA 9100376 SA 52346

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 06/02/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0259149	09-03-88	AU-B- 608769	18-04-91
		AU-A- 7791887	10-03-88
		JP-A- 63218627	12-09-88
EP-A- 0273366	06-07-88	AU-A- 8310887	30-06-88
		JP-A- 63273478	10-11-88